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HYPERTENSION AND THE RELATION BETWEEN BODY WEIGHT AND KIDNEY WEIGHT

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THE METABOLIC activity of an animal is related to its body weight. The greater the weight of an animal the greater will be its basal metabolism. But, as was discovered long ago and repeatedly confirmed, basal energy metabolism does not vary directly with simple body weight $W^{1.0}$, but with what Brody calls *metabolic effective body weight.* This is defined by W^b, where W represents body weight, b "represents the slope of the curve when plotted on logarithmically coordinate paper and is easily computed from the given data" (Brody, 1945). By this method the value of *b* was found to be 0.73 from data "including animals ranging in weight from mice to cattle and perhaps elephants" (Brody, 1945), (fig. 1).

The same general equation $y = a\,x^b$ has been successfully employed for relating the weight of different organs with body weight. (This equation is a parabola or a logarithmic equation, since on taking logarithms we obtain the equation $\log y = \log a + b \log x$ which has the same form as the linear equation $y = a + b\,x$).

In the case of the rat's kidney different workers have found for the exponent b values ranging from 0.6 to 0.72 (Walter and Addis, 1939; Foglia, Günther and Pinto, 1943; Braun-Menéndez, 1946a). This means that whatever the age of the rat the rate of growth of the kidney will be 0.6 to 0.72 times the rate of increase in body weight; or that an increase of 1 per cent in body weight is associated with a kidney weight increase of 0.6 to 0.72 per cent (fig. 2).

The discrete functions of the kidney (glomerular filtration rate, renal blood flow, tubular mass, etc.), are also related to approximately the 2/3 power of body weight. For the rat, Braun-Menéndez and Chiodi (1946) found less dispersion in their results when renal function was plotted against body surface ($W^{0.66}$) than when plotted against body weight. Smith (1937) calculated from the data in the literature the renal blood flow in cc per g of kidney per minute in rabbit, dog and man and found values of 3.2, 3.3 and 3.4 respectively. Adolph (1949) calculated from data obtained in different species by many authors the

equation relating various renal clearances to body weight obtaining for the exponents values from 0.69 for creatinine clearance to 0.89 for diodrast clearance (fig. 3).

In other words, kidney function and kidney weight both increase with approximately the 0.7 power of body weight. Kidney function

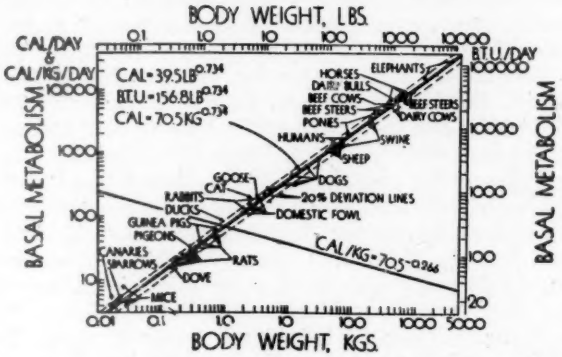

FIG. I. — *Basal metabolism in relation to body weight (Brody, 1945).*

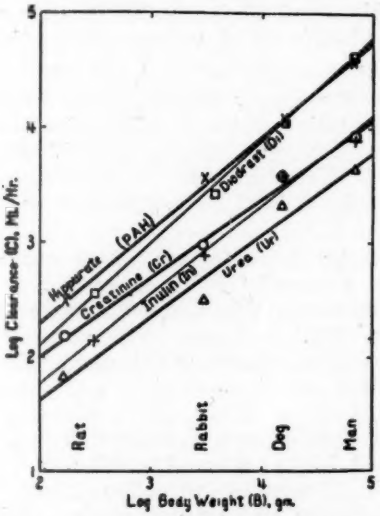

FIG. 2. — *Renal clearances in relation to body weight (Adolph, 1949).*

may therefore be predicted by the value of kidney weight and varies in parallel form. This statement is of course a rather crude simplification and only applies to what we agree in calling *normal* conditions. Kidney weight may increase in some pathological conditions without a corresponding increase in kidney function the same as the basal metabolism

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may increase without increase in body weight. The same holds for the relation between kidney weight (or kidney function) and body weight. In normal conditions kidney weight or function will be related to body weight according to the equation: kidney weight = a Body weight$^{0.7}$. But many physiological factors are able to cause a change in the rate of growth of the kidneys without a corresponding change in the rate of corporal growth. As a consequence of the action of those factors the weight of the kidney will be greater or smaller than the predicted weight: we speak then of hypertrophy or atrophy of the kidney. A protein rich diet (Watson and Lyon, 1906; Osborne and Mendel, 1924; MacKay, and Addis, 1925; Miller, 1925; Reader and Drummond, 1925; Anderson, 1926; Jackson and Riggs, 1926; MacKay, MacKay and Addis, 1928; Reid, 1944, 1947; Leathem, 1947; Braun-Menéndez and Ruíz-Guiñazú, 1951), the administration of crude anterior pituitary extracts (Selye, 1944) and especially the thyrotrophic (Hay, 1946; Hay, Seguin, Lariviere and Jensen, 1946) and somatotrophic hormones (Kochakian and Stettner, 1948); thyroid feeding or administration of thyroxine (MacKay and MacKay, 1931; MacKay, Smith and Closs, 1933; Swann, 1939; Selye, Stone, Nielsen and Leblond, 1945; Herlant, 1949; Braun-Menéndez, 1950) and testosterone (Korenchewsky, Hall and Burbank, 1939; Selye, 1939; Feyel, 1943; Ludden, Krueger and Wright, 1941; Selye, 1948; Kochakian and Stettner, 1948) all cause hyperthophy of the kidneys, having what is called a renotrophic effect.

On the other hand, a protein deficient diet (Braun-Menéndez and Ruíz Guiñazú, 1951), hypophysectomy (Smith, 1930, Selye, 1941; Foglia and Houssay, H., 1942; Fontaine and Veil, 1945; Fontaine, 1947; Braun-Menéndez, 1950), thyroidectomy (MacKay and MacKay, 1931; MacKay, Smith and Closs, 1933; Zeckwer, 1946; Braun-Menéndez, 1950) and castration in males (Leathem, 1948) cause a more or less marked atrophy of the kidney.

Interestingly enough those factors which cause a hypertrophy of the kidneys produce a parallel increase in kidney function. A high protein diet causes an increase in glomerular filtration and renal blood flow in rats (Dicker, 1948) and men (Pullman, Alving and Landowne, 1949); administration of anterior lobe extracts produces a great increase in glomerular filtration, renal blood flow and tubular excretory mass in dogs (Heinbecker, Rolf and White, 1943; White, Heinbecker and Rolf, 1947), especially so when growth hormone is injected (White, Heinbecker and Rolf, 1949, 1951); thyroid powder or thyroxine administration causes a rise in glomerular filtration, renal blood flow, tubular excretory mass and glucose reabsorption in dogs (Heinbecker, Rolf and White, 1943; Eiler, Althausen and Stockholm, 1944; Hare, Phillips, Bradshaw, Chambers and Hare, 1944; Handley, Moyer, Kennedy and Costa, 1951) and testosterone produced a marked increase in diodrast Tm in female dogs (Welsh, Rosenthal, Duncan and Taylor, 1942); results not confirmed by Richardson and Houck (1951).

On the other hand those factors causing an atrophy of the kidney produce a parallel decrease in kidney function. A protein deficient diet

causes a decrease in glomerular filtration, renal blood flow and tubular excretory mass in rats (Dicker, Heller and Hewer, 1946), dogs (Joliffe and Smith, 1931; Zucker, Cizek and Tompkins, 1950) and men (Pullman, Alving and Laudowne, 1949); hypophysectomy causes a very marked decrease in glomerular filtration, renal blood flow and tubular excretory mass in dogs (White and Heinbecker, 1940; White, Heinbecker and Rolf, 1941; White, Heinbecker and Rolf, 1942; Earle, de Bodo, Schwartz, Farber, Kurtz and Greenberg, 1951) and thyroidectomy produces some fall in glomerular filtration, renal blood flow and tubular excretory mass in dogs (White, Heinbecker and Rolf, 1947).

It is worthwhile to point out here that hypophysis, thyroid and testoids, the excess of which causes hypertrophy and hyperfunction of the kidneys and the absence of which produces an atrophy and hypofunction of the kidneys, have a definite action on the general metabolic activity of the body and are especially important factors in the regulation of protein metabolism.

We can summarize this presentation of facts by stating: 1) that kidney function varies with kidney weight, and, 2) that kidney weight (and kidney function) are in some sort related to the metabolic activity of the body. In normal conditions kidney weight and function are related to approximately the 0.7 power of the body weight; in other words they bear the same relation to body weight as the metabolic activity of the body. It would be equally truthful to say that kidney weight and function are directly correlated to the metabolic activity of the body. Those factors which increase the latter produce an increase in kidney weight and function, without a proportionate increase in body weight (renal hypertrophy and hyperfunction) and contrariwise those factors which decrease the metabolic activity of the body cause a disproportionately great decrease in kidney weight and function (renal atrophy and hypofunction).

COMPENSATORY HYPERTROPHY OF THE KIDNEY

When one of the kidneys is extirpated the remaining kidney increases in size and weight: the glomeruli and the tubular cells increase in size, in short a hypertrophy of the kidney takes place. But there is also some degree of hyperplasia; a few mitosis can be observed in the tubular cells during the first days following unilateral nephrectomy; nevertheless the number of nephrons does not change (see below). This phenomenon is generally designated as compensatory hypertrophy, a term which only means that after removing one kidney the remaining one grows at an abnormally fast rate.

The process of compensatory hypertrophy of the kidney has been studied by many authors since Rayer, in 1841 mentioned the fact, and data in the literature are rather contradictory with respect to the time in which this process is completed. Some authors give 6 to 8 weeks (Smith and Moise, 1927), others 80 days (Arataki, 1926), and some

even 4 months (Moise and Smith, 1926; Jackson and Shiels, 1927) as the time taken for the compensatory hypertrophy to be completed. Addis and Lew (1940) observe that at 20 days the curve begins to fall off and at 40 days it is nearly asymptotic. But none of these authors have taken into consideration the fact that the growth of the sole remaining kidney does not bear the same relation to increase in body weight as the growth of one of the two normal kidneys *in situ*.

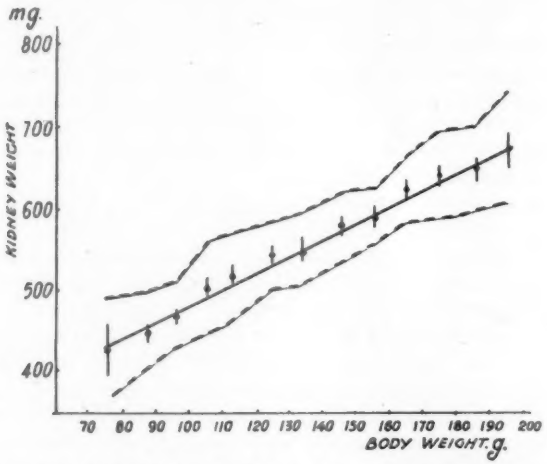

FIG. 3. — *Kidney weight in relation to body weight (from Braun-Menendez, 1946, a).*

We have studied the problem in the following way. The growth curve of one of the normal kidneys of albino rats from 70 to 200 g corporal weight was first established. A linear relation was obtained between these values which can be expressed (for this body weight range) by the equation $y = 2.06\,x + 277$ where y is the weight of one kidney in mg and x is body weight in g (fig. 3). A better fit is obtained applying the logarithmic equation $y = 12.27\,x^{0.71} + 164$ (Braun-Menéndez, 1946 a).

We then studied in normal rats of both sexes and in the puberal and adult stage the modification caused by the removal of one kidney on the growth of the remaining kidney. We observed that the weight of the remaining kidney increases rapidly during the 2 to 3 weeks following unilateral nephrectomy. No significant sex or age differences were observed. After this period of accelerated growth the sole remaining kidney continues to grow together with the whole organism; but as could be expected it grows at a slightly faster rate than one of the two kidneys of a normal animal. The relation between weight of the sole kidney and body weight can still be expressed by a linear equation but the slope of the curve and the additive constant are greater. The equation is now $y = 3.74\,x + 422$ (Braun-Menéndez, 1946 b).

Unilateral nephrectomy causes thus the transition from the rate of growth of a normal kidney to the sligtly greater rate of growth of the sole remaining kidney and in the intervening period of 14 to 21 days, during which the rate of kidney growth is greatly accelerated, the process of real compensatory hypertrophy is completed. We use the qualification

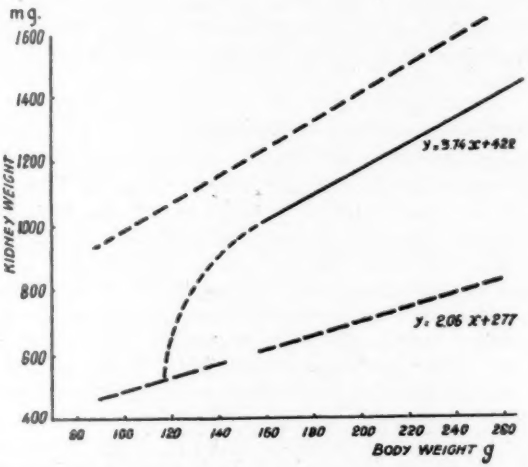

FIG. 4. — *Unilateral nephrectomy causes the transition from the rate of growth of the normal kidney ($y = 2.06\ x + 277$) to the slightly greater rate of growth of the sole remaining kidney ($y = 3.74\ x + 422$) (from Braun-Menendez 1946, b).*

of real because in a sense the slightly greater rate of growth of the sole remaining kidney observed during the rest of the life of the animal is also compensatory since only one kidney instead of two will have to subvene to the increasing demands of the growing animal. Not having considered this fact, the authors which have studied the problem concluded that the period of compensatory hypertrophy was of very long duration (fig. 4).

Much work has been done on the factors which may modify the course of compensatory hypertrophy of the kidney. In spite of some contradictory results (McQueen-Williams and Thompson, 1940; Winternitz and Waters, 1940; Gaarenstrom, 1942; Colonge, 1944) it has been repeatedly verified that in hypophysectomized animals (Selye, Martínez, Thomson and Collip, 1934; González, 1938; Fontaine and Veil, 1947; Braun-Menéndez and Houssay, H., 1949), the compensatory hypertrophy of the kidney after unilateral nephrectomy may be diminished but is far from being abolished. The same thing occurs in thyroidectomized rats (Braun-Menéndez, 1950).

We have seen that renal compensatory hypertrophy is only an acceleration of growth of the remaining kidney caused by the removal of the contralateral kidney. If to this renal growth-stimulating factor we add the action of other factors which are able to modify the growth of the normal kidneys the result will be an accentuation or an attenuation of the process of compensatory hypertrophy. The results are not always clear-cut because of the action of two or more factors which may be concurrent or discordant. In fact no information is obtained by studying the effect of these factors in compensatory hypertrophy which cannot be obtained by studying their effect on the relative growth of the normal kidney.

Degree of renal compensatory hypertrophy

The histological study of the remaining kidney after unilateral nephrectomy reveals a real tissular drama: during the first 6 days a great dilatation of the Bowman space and of the tubules is observed followed by a more gradual enlargement of the glomerular portion of the renal corpuscle and the tubular cells. At the end of 3 to 4 weeks the histological aspect of the kidney is again normal apart from the generalized hypertrophy. A few mitosis are observed especially between 2 and 8 days after the unilateral nephrectomy (Arataki, 1926; De Robertis and Poch, 1947; Rollason, 1946, 1949; Sulkin, 1949, 1950).

The new equilibrium is established when the sole remaining kidney reaches a weight representing approximately 80 % of the theoretical total renal mass (Braun-Menéndez, 1946 b). Why does the kidney stop growing acceleratedly at this point? Is it due to an incapacity to grow further? Is the weight of the sole remaining kidney an exact index of the amount of functional tissue? Is the functional capacity of the organ completely restored? These possibilities merit further discussion.

1) When the compensatory hypertrophy of the kidney is completed the organ has not lost its capacity for further growth. The administration of a protein rich diet, of pituitary extracts, of thyroxine or testosterone causes a further increase in weight of the sole kidney which may attain a weight representing 120 and more per cent of the theoretical total renal mass, that is, the single kidney may weight much more than the two kidneys of a normal untreated animal of the same corporal weight (Addis, 1948; Braun-Menéndez, unpublished data).

2) A possible explanation of the problem under discussion is that the weight of the organ is not, in this case, a reliable index of the amount of functional tissue present. It could be assumed that the supporting tissue, the excretory tubes and the blood vessels do not duplicate their weight while the glomeruli and tubules have reached a 100 per cent increase in weight. This is a very plausible explanation and one should conclude that the hypertrophy stops when the kidney reaches a weight representing 80 % of the theoretical renal weight because the essential functions of the organ have been restored.

3) But which are these essential functions? Are they the excretory functions which may be explored by the various clearances? Urea

clearance determinations in rabbits (Addis, Myers and Oliver, 1924) and in dogs (Rhoads, Alving, Hiller and van Slyke, 1934) show that even many months (4 to 22) after the unilateral nephrectomy the urea clearance does not surpass 80 per cent of the preoperative value. Van Slyke, Rhoads, Hiller and Alving (1934) found in one-kidney dogs that the renal blood flow was 71 per cent, the renal O_2 consumption 73 per cent and the urea clearance 60 per cent of the average of two-kidney dogs. We have studied the inulin clearance and diodrast Tm in rats 5, 20, 40 days and several months after unilateral nephrectomy, comparing the values found with those obtained in the same rats before nephrectomy (Braun-Menéndez and Chiodi, 1947). We found that as early as 5 days after unilateral nephrectomy the remaining kidney already is able to accomplish by itself about 70 per cent of the preoperative glomerular filtration and tubular excretion. Three months later the renal functions explored had reached only about 80 per cent of the normal preoperative values.

Cause and mechanism of renal compensatory hypertrophy

From the data in the literature and our own observations one can affirm that the nervous system has no intervention in renal compensatory hypertrophy nor in kidney growth. Denervation of the kidney does not modify the normal growth of this organ nor the process of compensatory hypertrophy. The factor controlling both must then be humoral.

The generally accepted hypothesis concerning the compensatory hypertrophy of the kidney —which could also be applied to its normal growth— is that the sudden elimination of 50 % of the renal mass determines an accumulation of metabolites in the blood (urea being the substance generally incriminated) which would stimulate the growth of the remaining kidney by demanding from it an excretory function double the normal.

Many facts are against this hypothesis: 1) Administration of great amounts of urea during many days may produce a slight hypertrophy of the kidneys (MacKay and Addis, 1928; Braun-Menéndez, 1950). It is impossible to deny that a greater elimination of urea and other metabolites, by increasing the work that the kidney must realize, may cause some degree of renal hypertrophy. But how can we explain the dramatic changes observed in the remaining kidney during the first days which follow unilateral nephrectomy which cannot be duplicated by the administration of enormous amounts of urea? Moreover, in compensatory hypertrophy the enlargement is more marked in the proximal convoluted tubules (Addis, 1948). If the osmotic work of the kidney to which Walter and Addis (1939) refer is the reabsorption of water against osmotic pressure this takes place in the distal tubule.

2) Oxygen consumption in the sole remaining kidney is about 73 per cent (Van Slyke, Rhoads, Hiller and Alving, 1934) of the sum of the oxygen consumption of the two kidneys in normal dogs. This increase in oxygen consumption is not due to the increased excretion of urea because the same authors found that a "10 fold increase in the blood urea content

and hence in the rate of urea excretion", caused no increase in oxygen consumption of the kidney.

3) "The apparent significance of these results is that not only the work directly involved in concentrating the urine, but also whatever processes are indirectly connected with it, taken together, consume so little of the total oxygen absorbed by the organ that maximal variations in the excretory work are without measurable influence on its total oxygen consumption. The overwhelmingly greater part of the energy produced must be utilized by the kidney for its own internal cellular processes not related to the external work which the organ is performing" (Van Slyke, Rhoads, Hiller and Alving, 1934). This conclusion is supported by many facts. Barcroft and Brodie (1905) and Glaser, Laszlo and Schürmeyer (1933) have calculated that the mechanical work which the kidneys do in concentrating and excreting the constituents of the urine is less than 1 per cent of the total energy produced by the organ as measured by its oxygen consumption. By the administration of urea and reduction of ingested fluids it is possible in the rat to obtain urine with 10 per cent urea in which case the osmotic work of the kidney is about 60 times greater than the work done when the urine has only 0.1 per cent urea. The oxygen consumption only increases 22 per cent (Dock, 1933). Human kidneys consume approximately 10 cc of 0_2 (Bradley and Halperin, 1948), per minute which represents more or less 50 cal and the thermodynamic work of excretion of the kidney of hydropenic subjects has been calculated in 0.6 cal/min (Rapoport et al., 1949). Moreover O_2 consumption by the kidney does not change appreciably even in conditions of maximal work such as saturation with paraaminohippurate, mannitol or water diuresis, etc. (Clark and Barker, 1951).

We may conclude that the increased excretory function demanded upon the sole remaining kidney cannot be the principal cause of its compensatory hypertrophy. Which are then the essential function of the kidney which determine its rapid growth when 50 per cent of the total renal mass is removed and the detention of this growth when it has reached a weight amounting to about 80 per cent of the preoperative total renal mass?

We are used to think of the kidney as an organ exclusively devoted to excrete the waste products of metabolism. But there is ample evidence that it also plays an important part in the metabolism of the body. There is evidence pointing out that the kidney participates in carbohydrate and lipid metabolism. The kidney is the locus of formation of urinary ammonia, which is formed from glutamine in the kidney by the enzyme glutaminase (Van Slyke, Phillips, Hamilton, Archibald, Fuchter and Hiller, 1943). Aminoacids and adenosine phosphate contribute ammonia in renal tissue as they do in other tissues, perhaps in larger proportions owing to the great deaminating power of this tissue (Peters and Van Slyke, 1946). The kidneys also participate in the synthesis of nitrogen products such as creatine. According to Borsook and Dubnoff (1941) only the kidneys can utilize arginine, converting it to guanidoacetic acid from which the liver can form creatine. However the kidneys

appear to be able to complete the synthesis of creatine (Borsook and Dubnoff, 1940). Blood from the renal vein contains less protein than blood from the renal artery (Gerbi, 1951). The kidneys have a very high proteolytic activity and they are probable concerned in the control of protein metabolism. The frequent association of fatty liver and degenerative kidney lesions suggest that both organs play a role in the synthesis of the nitrogen bases in the phosphatides.

Apart from these data the fact that the already mentioned factors which are able to modify so greatly the rate of growth of the kidney

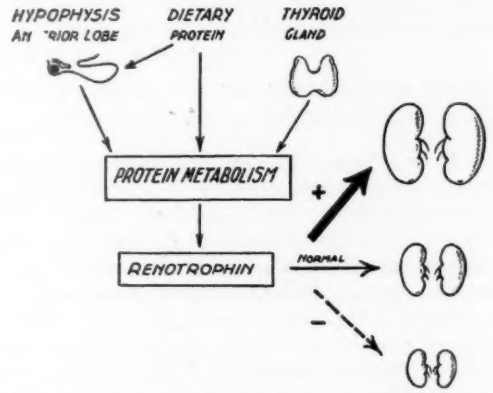

FIG. 5. — *Regulation of the size and functional capacity of the kidneys.*

(hypophysis, thyroid, androgens, protein richness of the diet) are also prominent in their influence on protein metabolism induced us to associate the so called compensatory hypertrophy with some disturbances in protein metabolism. This led us to formulate a working hypothesis which may be expressed as follows: *The size of the kidney and its functional capacity —which in normal conditions are more or less closely related— would be determined by the concentration in the blood of one or various substances, (probably some intermediary product of protein metabolism), which the kidney must eliminate or, more probably, transform and metabolize. When the production of these kidney growth stimulating substances (which for greater convenience we may designate renotrophin) increases, the kidney increases in size and function until a new equilibrium is reached between production and destruction, transformation or elimination of the said substances.* Thus when a protein rich diet, pituitary extracts, thyroid hormones or testosterone is given —factors which increase the production of renotrophin— the kidneys grow. When the administration of these factors is suspended the kidneys regain their previous normal weight. Contrarywise a protein poor diet, hypophysectomy or thyroidectomy would cause a reduction in renal weight because of a lesser production of renotrophin by the organism (fig. 5).

If the nutritional and hormonal conditions being constant, the renal substance is artificially reduced (unilateral nephrectomy, partial nephrectomies, induced renal lesions, etc.), the concentration of renotrophin should increase and this increase stimulates the growth of the remaining kidney tissue increasing its functions until the equilibrium between formation and destruction of renotrophin is again reestablished.

We designate renotrophin the factor which stimulates kidney growth by increasing the demands for its work and we believe that factor to be of humoral nature and of metabolic origin. When we speak of renal function we refer not simply to the excretory function of the kidney but specially to that non identified chemical work which would be regulated by the production and blood level of renotrophin.

COMPENSATORY HYPERTROPHY OF THE KIDNEYS AND ARTERIAL EXPERIMENTAL HYPERTENSION

Our interest in the intriguing and obscure phenomenon of compensatory hypertrophy of the kidney arose really from our work in the field of experimental arterial hypertension. There is an observation in this field which could never receive an adequate explanation. The constriction of one renal artery, the other kidney being intact is followed in dogs (Goldblatt, Lynch, Hanzal and Summerville, 1934; Goldblatt, 1937), and in rabbits (Pickering and Prinzmetal, 1938) by a rise in blood pressure. But this rise in blood pressure is less marked (Fasciolo, 1938, 1939) and less rapid (Fasciolo, 1938, 1939), than when clamps are applied to both kidneys and is usually of a transient nature (Goldblatt, 1937, 1938, 1940). But if once the blood pressure has returned to its preoperative level, the intact kidney is removed there is a prompt rise of blood pressure to higher levels and a permanent hypertension ensues (Blalock and Levy, 1937; Fasciolo, 1938; Katz, Mendlowitz and Friedman, 1938). Fasciolo (1938) concluded from these observations that the normal kidney exerted a "protective" action in renal experimental hypertension.

The phenomenon described has been interpreted in many different ways. The mechanism of this hypothetic "protective" action could be: 1) the destruction, excretion or inactivation by the normal kidney of the hypertensive substance elaborated by the ischemic kidney; 2) the elaboration and secretion by the normal kidney of a substance which destroys, inactivates or antagonizes the pressor substance formed by the ischemic kidney. I will not discuss here the evidence in favour or against these two possible explanations (for discussion see Braun-Menéndez, Fasciolo, Leloir, Muñoz and Taquini, 1946). Whichever the concept adopted the fact that the removal of the intact kidney leads to a greater elevation of the blood pressure and the establishment of a permanent hypertension indicates that the normal kidney in some manner compensates for the deleterious effects of the ischemia in the other kidney.

The observation first recorded by Pickering and Prinzmetal (1938) that after unilateral renal ischemia in the rabbit, the clamped kidney

was of normal consistency but very small while the intact kidney was enlarged led us to explain the so-called protective action of the normal kidney by associating our hypothetic renotrophin with the genesis of renal hypertension. According to this hypothesis: *whenever some impediment exists for the kidney to grow in size and function in order to maintain renotrophin at its normal level hypertension appears.* The rise in blood pressure will produce an increase in renal blood flow and hence in renal function. Up to a certain point hypertension would thus be a compensatory mechanism leading to the reestablishment of the equilibrium between renotrophin production and destruction.

We will examine at the light of the present hypothesis some experimental facts which seem to be best explained by it, beginning with the phenomenon we have just described.

I) *Conditions in which the production of renotrophin* (renal growth stimulating factor) *remains constant and renal functional mass is decreased.*

Under this heading we wil discuss those experimental conditions in which no change occurs in the production of renotrophin, i.e., in the functional demands upon the kidney, and hypertension is the result of a reduction in kidney functional mass *.

1) *Reduction of renal blood supply.* — Constriction of the renal artery of one kidney, the contralateral kidney remaining intact, would produce a disproportion between the work demanded upon the kidney which remains constant and its functional capacity limited by its reduced blood supply. As a consequence of the reduction in the ischemic kidney's function the concentration of renotrophin in the blood should increase: the result being an increase in blood pressure ** and a stimulation of the growth of the intact kidney. If the growth of the intact kidney is unhampered, in due time this kidney which is now hypertrophied would take over the work the ischemic kidney is unable to perform, thus reestablishing the equilibrium between renal functional mass and renotrophin. The blood pressure falls to normal levels. Thus can be explained the so-called "protective" action of the normal kidney.

If at this time the intact kidney is removed a desequilibrium occurs again between production of renotrophin which remains constant and renal function which has been reduced by sudden reduction of renal mass. The concentration of the former in the blood would increase. The kidney with the constricted artery cannot increase its functional mass because its blood supply is reduced by the mechanical obstruction. The blood pressure rises to higher levels than in the previous case and remains high permanently. A new equilibrium has been established between

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* We are referring here to the renal function connected with the regulation of renotrophin.

** We shall not discuss here the mechanism or mechanisms responsible for the resulting hypertension which have been the object of previous work (see Braun-Menéndez et al., 1946).

production of renotrophin and renal function involving a rise in blood pressure and perhaps a higher concentration of renotrophin in the blood (fig. 6).

If instead of removing the intact kidney its artery is also constricted thus reducing its blood supply, the result will be the same: the new equilibrium between renotrophin and reduced kidney function will involve also a rise in blood pressure and a higher concentration of renotrophin in the blood.

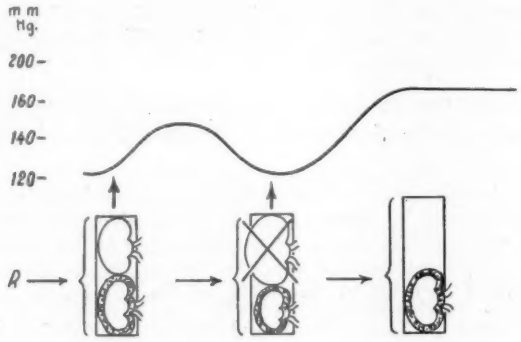

FIG. 6. — *Diagramatic representation of the so-called protective action of the normal kidney and the effect of its extirpation.*

2) *Reduction of the amount of functioning renal tissue.* — Practically all the methods devised for the production of experimental arterial hypertension involve a reduction in the amount of functioning renal tissue. As we have seen partial resection of the kidney or partial destruction of its parenchyma will be followed by compensatory hypertrophy of the remaining healthy tissue. If reduction of renal parenchyma is extreme the animals die in renal excretory insufficiency. If the hypertrophy of the remaining healthy renal tissue is unhampered normality is restored. Between these two extremes hypertension occurs.

Reduction of the amount of functioning renal tissue may be accomplished by: a) surgical reduction of the renal parenchyma; b) ligation of the renal artery or its branches; c) lesions of the renal tissue by biological, chemical or physical means.

a) *Surgical reduction of the renal parenchyma.* — Unilateral nephrectomy may cause a slight and transient elevation of blood pressure but hypertension only occurs if the remaining kidney is the seat of pathological lesions (Grollman and Halpert, 1949), which would limit or prevent compensatory hypertrophy. Unilateral nephrectomy plus partial resection of the other kidney thus reducing the renal substance to about 20-30 per cent causes a pronounced and chronic hypertension in the

rat (1). The scarring from the surgical operation is probable the factor which limits the compensatory hypertrophy of the remaining renal tissue. In the dog, reduction of renal mass has produced only slight and transitory rises of pressure (2), even if some of the branches of the renal artery were also ligated (3). This species difference may be due to the fact that experiments are generally made on rats during their growing age while adult dogs are used (see effect of body growth).

b) *Ligation of the renal artery or of its branches.* — Ligation of both renal arteries (4), of one main renal artery (5), or of its branches (6) may cause a rise in blood pressure which is slight and temporary or will cause the death of the animal in uremia. It appears reasonable to assume that if enough healthy tissue is left its hypertrophy will compensate in due time for the lost tissue; if the destruction has been too great the animal dies in renal insufficiency.

c) *Lesions of the renal tissue.* α) *Nephrotoxic substances.* — The effect of nephrotoxic substances such as uranium (7), or lead salts (8), sodium (9) or potassium oxalate (15), trypsin (11), streptococcus (12) or diphteria (13) toxin has been tried but elevation of blood pressure was not produced consistently. Here again, destruction of renal tissue is compensated by the unhampered compensatory growth of the remaining normal tissue. If the destruction of parenchyma is too great renal insufficiency will appear leading to the death of the animal.

The injection of anti-kidney serum produces renal lesions which reproduce those found in glomerulonephritis and also an elevation of blood pressure (14). Glomerular lesions, by limiting the blood supply to

(1) Chanutin and Ferris (1932); Chanutin and Barksdale (1933); Wood and Ethridge (1933); Rytand and Dock (1935); Díaz and Levy (1939); Harrison, Grollman and Williams (1940); Griffith and Ingle (1940); Jensen, Corwin, Tolksdorf, Casey, Bamman (1941); Gómez-Salazar (1942).

(2) Allen, Scharf and Lundin (1925); Mark (1925, 1928); Pässler and Heinecke (1904).

(3) Cash (1924); Mark and Giesendörfer (1930); Hantschmann (1931); Ferris and Hynes (1931).

(4) Katzenstein (1905); Cash (1926); Goldblatt (1937); Dicker (1937 b); Blalock and Levy (1937); Winternitz, Mylon, Waters and Katzenstein (1940).

(5) Friedman and Wachsmuth (1930); Hartwich and Hessel (1932); Wolf and Heinsen (1935); Konzett and Unna (1937); Verney and Vogt (1943); Robbers and Westenhoeffer (1939); Winternitz, Mylon, Waters and Katzenstein (1940).

(6) Janeway (1908, 1913); Mark (1928); Hartwich (1930); Friedman and Wachsmuth (1930); Wolf and Heinsen (1935); Konzett and Unna (1937); Verney and Vogt (1938); Loomis, 1946.

(7) Beckman (1925); Domínguez (1928).

(8) Griffith and Lindauer (1944).

(9) Arnott and Kellar (1935).

(10) Israel (1938).

(11) Friedman and Katz (1938).

(12) Dicker (1935); Dick (1945).

(13) Beckmann (1925).

(14) Masugi (1934); Arnott, Kellar and Matthew (1936); Hamori and Koranyi 1938); Kay (1940 a, b, 1942); Kay, Lucchesi and Rutherford (1941) in rabbits; Smadel (1937); Smadel and Farr (1937); Knowlton, Stoerk, Seegal and Loeb (1946); Knowlton, Loeb, Stoerk and Seegal (1947); Loeb, Knowlton, Stoerk and Seegal (1949); Knowlton, Loeb, Seegal and Stoerk (1949) in rats.

the renal parenchyma may be the limiting factor of compensatory growth of the remaining healthy tissue. We shall see later on, that animals with this type of renal lesions react more readily and with greater elevation of blood pressure to certain factors which increase the demand upon the kidney.

Irradiation with roentgen rays [15] multiple renal emboli [16], passive congestion of the kidney by venous obstruction [17], occlusion of the ureters [18], etc., are simply different methods producing the same effect, i.e., lesions of the renal parenchyma, and whether they produce or not hypertension will depend on one hand on the degree of reduction of renal functioning tissue accomplished and the possibility of compensatory hypertrophy of the remaining normal tissue on the other.

The production of hypertension by renal compression and perinephritis merits special consideration. This method first employed by Page (1939 a and c) in dogs, cats and rabbits consists in the envelopment of the kidneys in cellophane, silk or other materials [19]. The tissue reacts and in 2 or 3 weeks a thick perirenal scar is formed as the result of the perinephritis induced by the foreign material. All these methods produce similar effects: 1) a reduction in renal tissue caused by the perinephritic reaction and which involves the outer layers of the cortex, and; 2) the envelopment of the kidney in an inextensible cast which prevents its growth. To obtain a chronic more or less benign hypertension the best procedure is to operate on one kidney and after some time remove the intact one. As a result of the rigid cast which surrounds the remaining kidney compensatory hypertrophy cannot occur and hypertension results. Incidentally it is worthwhile mentioning that the stimulus to renal growth is such that if a hiatus however small exists in the envelop, the kidney substance will form an hernia and break through the opening. The second best is to provoke perinephritis in both kidneys, but this very frequently causes a malignant hypertension with pronounced renal insufficiency.

In the rat, unilateral perinephritis causes hypertension in a variable proportion of cases although the opposite normal kidney is intact. But in these cases, histological study of the latter shows glomerular and tubular lesions which may be caused either by the transient hypertension

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(15) Hartman, Bolliger and Doub (1929); Page (1936).

(16) Maegraith and McLean (1938).

(17) Pedersen (1927); Bell and Pedersen (1930); Braun-Menéndez (1932); Dicker (1937 b); Friedberg (1941, 1944).

(18) Rautenberg (1910); Hartwich (1930); Dicker (1937 c); Harrison, Mason, Resnik and Rainey (1936); Blalock and Levy (1937); Megibow, Friedberg, Rodbard and Katz (1940); Megibow and Katz (1941); Megibow, Katz and Rodbard (1942); Goldblatt and Kahn (1940, 1941).

(19) *Cellophane:* Hermann, Jourdan and Vial (1940); Friedman, Jarman and Klemperer (1941); Schroeder (1942); Gómez-Salazar (1942). *Collodion*: Greenwood, Nassim and Taylor (1939); Herrman, Decherd and Erhard (1941); Page, Patton and Ogden (1941); Dell'Oro (1942 a, b); Gaudino (1944); Braun-Menéndez and Martínez (1949); Gómez-Salazar (1942); Magaldi (1945). *Silk:* Kempf and Page (1942); Grollman and Williams (1942). ***Rayon:*** Gómez-Salazar (1942). *Cotton*: *Cromartie* (1943). *Latex*: Abrams and Sobin (1947). *Plastics*: Rau (1950).

following unilateral perinephritis or perhaps by the action of substances secreted by the compressed kidney. In our experience, hypertension following unilateral perinephritis is more frequent when the operation is made in young rats and when a growth promoting diet is used. We will return on the significance of these factors later on. The method devised by Grollman (1944) of passing a cotton tape or thread over the pole and body of the kidney and drawing it taut, apart from causing ischemia by compression and some destruction of renal tissue also prevents or at least limits the compensatory hypertrophy of the kidney. In this case also the best results are obtained by placing the ligature in one kidney and then removing the opposite.

Various sterols have been shown to be able to give rise to renal lesions and hypertension [20] but the most effective and best studied is desoxycorticosterone [21]; administration of this steroid in rats and dogs produces nephrosclerosis and a chronic sustained hypertension when sodium salts are present in the diet [22]. According to Selye (1950) the first morphologic changes detectable are distension of the convoluted tubules, with some hypertrophy and hyperplasia of the lining cells. Eventually, however, glomerular hyalinization and hyalin necrosis of the arterioles ensues. When the glomerular circulation becomes seriously damaged by hyalinosis the corresponding tubules (the proximal convoluted tubules) lose their eosinophilia and brush border while their lumina disappear. According to this description the drug causes destruction of renal parenchyma and due to glomerular and arterial lesions hindrance to the compensatory hypertrophy of the remaining healthy tissue. A very interesting fact is that in those conditions in which the kidney mass is further reduced the animals are strongly "sensitized" to the action of desoxycorticosterone. Thus unilateral nephrectomy [23], nephritis induced by antikidney serum [24], renal hypertension [25] and diabetes [26] sensitize the animals to the toxic and hypertensive effects of desoxycorticosterone.

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[20] *Cholesterol:* Schonheimer (1942); Lowenthal (1926).
Vitamin D_2: Appelrot (1933); Handovsky (1937); Ham (1940).

[21] Selye (1942 a, b 1943); Selye and Stone (1943); Selye, Hall and Rowley (1943); Selye and Pentz (1943); Selye and Hall (1943, 1944); Selye and Rowley (1944); Rodbard and Freed (1942); Briski, Stokes, Reed and Mrazek 1943).

[22] Selye, Stone, Timiras and Schaffenburg (1949); Friedman, Friedman and Polley (1948); Braun-Menéndez and Prado (1950).

[23] Selye and Hall (1943); Selye and Pentz (1943).

[24] Knowlton, Loeb, Stoerk and Seegal (1947); Knowlton, Stoerk, Seegal and Loeb (1946).

[25] Rodbard and Freed (1942); Knowlton, Loeb, Stoerk and Seegal (1947); Knowlton, Stoerk, Seegal and Loeb (1946); Knowlton, Loeb, Seegal and Stoerk (1949); Braun-Menéndez (1951).

[26] Braun-Menéndez and Martínez (1949).

[27] Chanutin (1934); Chanutin and Ludewig (1936 a, b); Chanutin and Ludewig (1939, a, b, c).

II) *Conditions in which the production of renotrophin increases and the increase in renal functional mass is impeded.*

Under this heading we will discuss those experimental conditions in which the functional demand upon the kidney i.e. production of renotrophin, increases and hypertension results because the kidney is unable to increase its functional mass due to the presence of lesions or mechanical obstacles. Among the factors which increase the demands upon the kid-

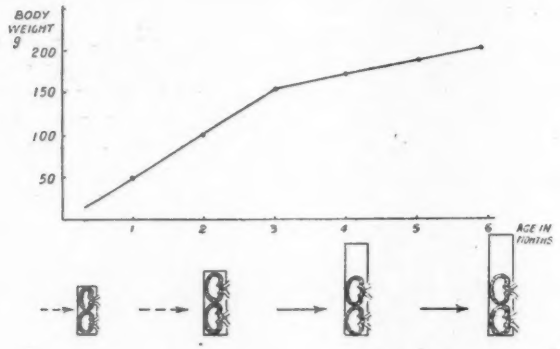

FIG. 7. —*Diagramatic representation of the effect of growth. The demands upon the kidney increase without this organ being able to increase its size and functin.*

ney (production of renotrophin) we shall mention: a) growth; b) anterior lobe of the hypophysis; c) thyroid; d) protein rich diet.

a) *Growth.* — In the rat growth in body weight and body length is practically continuous until they reach an age of 18 to 20 months, which is very near the age of senescence. The growth is rapid during the first 5 months of life and then slows down. In the dog, on the other side, growth is more similar to human growth, i.e. it stops when adult age is attained. This difference in growth characteristics may explain many of the differences observed in these two species with regard to their response to the experimental conditions causing hypertension. Most of the experiments made on rats are done during their maximal growing age; on the other hand, experiments on dogs are made when their growth has ceased.

Growth is accompanied by an increased demand upon the kidney and consequently by a proportionate increase in renal weight and function. The increased demand due to growth of the animal is gradual and progressive. Some experimental procedures which reduce the functioning renal tissue and/or limit renal growth may not cause an immediate rise of blood pressure in the rat; hypertension may appear

some time later as the animal grows and the demands upon the kidney increase without this organ being able to increase its size and function (fig. 7).

A very good example of this situation is hypertension following choline deficiency. If rats are kept on a choline deficient diet for a few days during early life and survive a severe acute attack of subcapsular hemorrhages, they develop hypertension which appears only 4 to 7 months later (Best and Hartroft, 1949). The pathogenesis of the renal lesions were described by Hartroft (1948): the initial lesion consists in the formation of droplets of fat within the epithelial cells of the proximal convoluted tubules of the cortex associated with swelling of the affected nephrons. Tubular swelling produces obstruction of the cortical capillary plexus with resultant tubular ischemia and necrosis. Proximal to the site of capillary obstruction the cortical vessels become enlarged and rupture into and beneath the renal capsule. The final result is a reduction of the renal vascular plexus of the cortex and the destruction of nephrons with obvious reduction of the renal functional tissue. There is also fibrotic organization in the areas of capsular and subcapsular hemorrhages and of the necrotic tissue just beneath the capsule which may be able to prevent or limit further growth of renal tissue.

The experimental data show that for hypertension to appear the renal damage must be severe and the animal's growth optimal (Best and Hartroft, 1949). If the rate of body growth is retarded (choline-deficient diet, caloric or protein poor diet, etc.), the animals will not become hypertensive (Sobin and Landis, 1947; Handler and Bernheim, 1950 b). On the other hand, a diet which promotes body growth and especially a protein-rich diet (which especially increases the demands on the kidney) will favour the appearance of hypertension (Best and Hartroft, 1949; Handler and Bernheim, 1950 b). Renal decapsulation perfomed one week after the acute episode of hemorrhagic kidneys "prevented the hypertension and performed 12 weeks after abolished the already established hypertension" (Handler and Bernheim, 1951). This may be explained by assuming that the fibrotic capsule of the kidneys is limiting the growth of the renal parenchyma. Nevertheless, as pointed out previously, this is not the only factor responsible for hypertension there being an appreciable reduction of renal functional tissue. The administration of ACTH to these rats after decapsulation restores hypertension (Handler and Bernheim, 1951), in the same manner as it restores hypertension of rats from which 3/4 of their renal tissue has been removed and in whom hypertension has been prevented by feeding a low protein diet (Handler and Bernheim, 1950 a, c).

The effect of growth on the appearance of hypertension caused by renal lesions explains many facts which have not yet received adequate explanation.

Drury (1938) produced hypertension in growing rabbits by means of a loop which kept the blood supply to the kidney from increasing as the work demanded from it increased. We have followed the course of hypertension due to unilateral perinephritis in hundreds of rats. In most

cases there appears a primary and transient rise of blood pressure during the first month of observation which is observed in a fairly high percentage of cases (50 to 70 %). In most of the animals the blood pressure returns to normal (1) and then in a small percentage (± 10 %) a gradual increase in blood pressure to hypertensive levels develops in a period of 2 to 4 months; a growth promoting diet favors the appearance of hypertension (Braun-Menéndez, unpublished data). If in these hypertensive animals the perinephritic kidney is removed, the blood pressure may fall to normal, may not change or may become irregular (Wilson and Byrom, 1941; Patton, Page and Ogden, 1943). The persistance of hypertension is probably due to the histological lesions present in the intact kidney (Wilson and Byrom, 1941). Gaudino (1944) found in some rats that following the post operative normalization of the blood pressure in some cases a slow progresive increase in blood pressure occurs which at the end of 4-8 months reaches the preoperative level. It is safe to assume that the remaining non operated kidney with vascular and cellular lesions caused by the pre-existing hypertension was able to cope with the function demanded at the moment of the operation; but being unable to grow at a rate proportional to body growth a gradual and progressive desequilibrium developed between body demands and renal function resulting in hypertension.

b) *Anterior lobe of the hypophysis.* — We have seen previously that the administration of the hormones of the anterior lobe of the hypophysis has a renotrophic action and causes a marked increase in renal function. This action we interpreted as due to an increased production of renotrophin. Selye (1944, 1946) found that the injection of impure pituitary extracts produces in the rat nephrosclerosis and hypertension (Prado et al., 1947; Dontigny et al., 1948; Masson, Corcoran and Page, 1949). These actions are greatly augmented by the administration of excess NaCl and are prevented by decreasing the protein concentration of the diet to 15 % (Dontigny et al., 1948). Selye (1951 a) has shown that the growth hormone is responsible for these actions and he believes that it acts through the adrenal, by liberating some desoxycorticosterone-like mineralocorticoids. Against this view are the following facts: 1) adrenalectomy prevents the renal lesions caused by anterior lobe extracts but not so the hypertension (Selye, 1951 b) · 2) the lack of correlation between adrenal weight and blood pressure level in rats given anterior pituitary extracts (Masson, Corcoran and Page, 1949).

The exact mechanism of the hypertension induced by anterior lobe extracts is still to be elucidated but it could be explained by a combination of two factors: an increase in renotrophin causing increased demands upon the kidney and a noxius action upon the kidney limiting its functional capacity and/or its growth. It is noteworthy that a high protein diet, the administration of thyroxine (Selye, Stone, Nielsen and Leblond,

(1) Rather (1950) concludes that the development of hypertension is independent from the total mass of functioning renal tissue. But as his rats were sacrified only 50 days after renal constriction and heterolateral nephrectomy, he was probably dealing with this initial and transitory rise in blood pressure.

1945) on one hand and unilateral nephrectomy (Selye et al., 1945) on the other, increase the ability of anterior lobe preparations to produce renal lesions and hypertension.

c) *Thyroid.* — It has been already mentioned that the production of renal lesions and hypertension by anterior pituitary preparations and desoxycorticosterone are facilitated by thyroxine administration (Selye, 1946). Page and Sweet (1937) found that the oral administration of thyroid (0.6 to 1.4 g per day) to hypertensive dogs in which hypophysectomy had reduced the hypertension, produced moderate rises of blood pressure. No study has been published on the action of thyroid feeding or thyroxine administration on the level of blood pressure in experimental hypertension or the incidence of hypertension after the various experimental procedures which cause renal involvement. Selye (1951 c) obtained nephrosclerosis and hypertension in rats to which 2 mg per day of thyroxine were administered.

d) *High protein diet.* — Chanutin and collaborators ([27]) carried out an extensive study of the action of protein diets on the kidney and systemic circulation of rats. In normal rats renal hypertrophy developed which was proportioned to the amount of protein in the diet, but no rise of blood pressure occurred. In rats with subtotal nephrectomy the incidence of hypertension was greater in the animals fed high meat diets (Chanutin and Ludewig, 1939 c).

High protein diets induce renal changes in the rabbit (Newburgh, 1919; Newburgh and Clarkson, 1923; Nuzum, Osborne and Sansum, 1925; Nuzum, Seegal, Garland and Osborne, 1926; Anderson, 1926) and may cause hypertension (Nuzum et al., 1926).

In hypertensive dogs, Verney and Vogt (1938) observed definite rises of blood pressure when fed meat plus salt or meat and urea for 1 to 7 days. Similar changes were not observed in normal animals or in those with subtotal nephrectomy. Maclachlan and Taylor (1940) confirmed these results. Wood and Cash (1939) using diets observed similar rises when the diet produced an increase of body weight. On the other hand Philipsborn, Katz and Rodbard (1941) obtained this elevation in only 2 of 14 dogs studied and Alpert and Thomas (1943) and Guerrant, Scott and Wood (1943) and Page and Lewis (1949) were unable to demostrate any effect of high protein diets in hypertensive dogs.

The observation of Wood and Cash (1939) may explain some of the contradictory results. These authors found that regardless of the nature of the diet, gain and loss in weight was associated in hypertensive animals with a rise or fall in blood pressure. Handler and Bernheim (1950 a) working on rats rendered hypertensive by subtotal nephrectomy or choline deficiency (Handler and Bernheim, 1950 b) found that the dietary levels of protein, calories and salt exert independently a profound influence on systolic blood pressure. The higher the protein level the higher the blood pressure. These authors report the important observation that urea feeding did not elicit a rise in pressure equal to the feeding of equivalent protein and conclude that it is unlikely that the effect of dietary protein on the blood pressure can be attributed merely to the

increase in osmotic work necessary to excrete the urea metabolized on a high protein diet.

The effect of high protein in the diet on the blood pressure of animals with renal hipertension may be explained as due to an increased production of renotrophin which increases the demands upon kidneys limited in their functional capacity and in their ability to hypertrophy.

III) *Conditions in which the production of renotrophin is decreased.*

We have reviewed in the preceding chapter the effect on the blood pressure of experimental animals of conditions in which the production of the hypothetic renotrophin increases and there is some impediment for the increase in renal mass. Body growth, the administration of anterior-pituitary extracts or thyroid feeding, a high protein diet are all factors which in normal conditions cause hypertrophy and hyperfunction of the kidneys; when due to experimental procedures the normal response to these factors is impeded an increase in blood pressure results.

If increasing the demands upon the kidney (increased renotrophin) causes a rise of blood pressure in the conditions described, a decrease of the production of renotrophin should have an inverse effect. a) Hypophysectomy; b) thyroidectomy, and c) a protein low diet are known to cause atrophy of the kidneys. We shall review the effect of these factors on the blood pressure of animals with experimental renal hypertension.

a) *Hypophysectomy.* — In dogs made hypertensive by partial constriction of the renal artery, Page and Sweet (1937) observed that hypophysectomy caused the blood pressure to fall. In some animals the blood pressure remained slightly elevated while in others it became entirely normal or even fell below the normal level. We have also observed a fall of blood pressure to normal levels after complete hypophysectomy in two chronically hypertensive dogs (Braun-Menéndez et al., 1943).

When the pressure is lowered as a result of hypophysectomy, tightening the clamps on the renal arteries or the administration of thyroid substance will cause further elevation of pressure (Page and Sweet, 1937). Hypophysectomy performed a month or more before the renal arteries were constricted did not prevent the development of renal experimental hypertension (Page and Sweet, 1937; Fasciolo, 1939; Goldblatt, Braden, Kahn and Hoyt, 1942). These results are difficult to interpret. On one hand the presence of the anterior lobe of the hypophysis seems indispensable to maintain the high blood pressure level produced by constriction of the renal arteries and on the other its absence does not prevent the hypertension resulting from constriction of the renal arteries.

But these facts can be made to fit into the hypothesis which we are considering. In the dogs rendered hypertensive by constriction of the renal arteries the condition is that reviewed in our first chapter: a reduction of renal functional mass with constancy of renotrophin production. If the production of renotrophin is reduced by hypophysectomy the desequilibrium established by constriction of the renal arteries disap-

pears and with it the rise in blood pressure. But if then we further reduce renal functional mass (by further renal artery constriction) or increase the production of renotrophin (by thyroid feeding) the desequilibrium again appears and with it the rise in blood pressure (fig. 8).

In the normal animal hypophysectomy causes a reduction of renotrophin production and with it a parallel reduction in renal functional mass. But if we then further reduce the latter by constriction of the

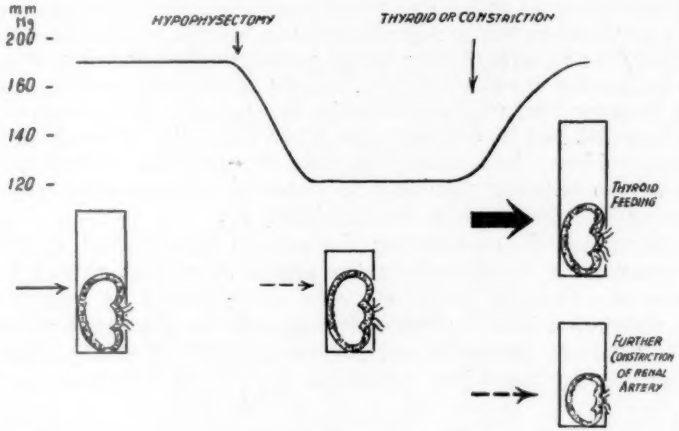

FIG. 8. — *Diagramatic representation of the effect of hypophysectomy in renal hypertensive dogs followed by thyroid feeding or further constriction of the renal artery.*

renal arteries the situation is the same as in the normal animal and hypertension develops.

The effect of hypophysectomy is surely more complex: through its influence on the adrenals it may affect the entire circulatory system and the metabolism of water and electrolytes. In the rat, for instance, ACTH corrects the hypotension caused by hypophysectomy in normal animals (Braun-Menéndez and Foglia, 1944), or the fall of pressure in hypertensive animals (Anderson, Page, Li and Ogden, 1944). In order to simplify the issue I have purposely ommited the consideration of this and other complications.

b) *Thyroid.* — Thyroidectomy in the dog does not prevent or cure hypertension produced by constriction of the renal arteries (Glenn and Lasher, 1938; Katz, Friedman, Rodbard and Weinstein, 1939). Since these early observations the thyroid was dismissed as a possible factor in experimental renal hypertension and curiously enough nobody has tried the effect of thyroidectomy on the hypertensive rat. As extirpation of the thyroid gland produces marked atrophy of the kidney (Braun-Menéndez, 1950), one would expect an effect on hypertension similar to that of hypophysectomy. We have recently performed such operation in

a few rats with chronic hypertension due to perinephritis and have obtained a reduction of the blood pressure to normal (unpublished observations). The explanation of this species differences is easy: thyroidectomy in the dog brings about only very mild manifestations of thyroid insufficiency while in the rat the syndrome is well developed.

c) *Low protein diets.* — The work of Handler and Bernheim (1950, a, b, c, d), has shown the importance of dietary protein in the maintenance and development of hypertension due to subtotal nephrectomy or choline deficiency in rats. The blood pressures of rats transferred from chow to low-protein ration fell promptly to normal levels. Urea added to a low protein ration exerted a slight pressor effect but it was not sufficient to account for the observed effects of dietary protein. The addition of tyrosine and phenylalanine in the amounts present in a 50 per cent casein diet had no effect on the blood pressure. These authors have presented evidence indicating that this phenomenon is largely due to failure of the anterior pituitary to elaborate adrenocorticotrophic hormone in rats on low protein rations (1950 c).

Incidentally when analyzing the effects of different diets on the blood pressure of hypertensives the effect on weight should be taken into account. Slowing of growth rate or reduction of weight due to caloric restriction has a profound influence on the blood pressure of hypertensive rats (Handler and Bernheim, 1950 a) and perhaps other animals including man (for references see Brozek, Chapman and Keys, 1948).

Is renotrophin a pressor substance?

We have examined the experimental facts which seem to be best explained by the hypothesis that renotrophin, the rate of production of which regulates kidney size and function, is related in some way with the development of renal hypertension. According to this hypothesis, whenever the kidney is unable to keep renotrophin at its normal level, hypertension appears.

We shall not discuss here the mechanisms which lead to hypertension but the question must be raised whether renotrophin may be a pressor substance. Some experimental facts seem to point in favor of the affirmative.

a) *Hypertension in nephrectomized animals.* — In 1947 while studying with von Euler the effect of parabiosis on blood pressure we came upon the unexpected fact that following total nephrectomy about 30 per cent of our rats developed hypertension (Braun-Menéndez and von Euler, 1947) in the short period of survival (2 to 4 days). We found a close correlation between the incidence of hypertension and the increase in blood and extracellular fluid volume (Braun-Menéndez and Covián, 1948) and ascribed to this factor a definite role in the causation of the blood pressure rise (Braun-Menéndez, 1951). But the possibility that nephrectomy caused the accumulation of a pressor substance in the blood was not disproved nor dismissed.

Grollman, Muirhead and Vanatta (1949) obtained a much greater survival time in nephrectomized dogs by means of a sodium and potassium poor diet and the application of the artificial kidney or intermittent peritoneal lavage (Grollman, Turner and MacLean, 1951). In all the animals maintained alive for periods as long as 70 days there was an elevation in blood pressure to hypertensive levels, without change in blood volume,

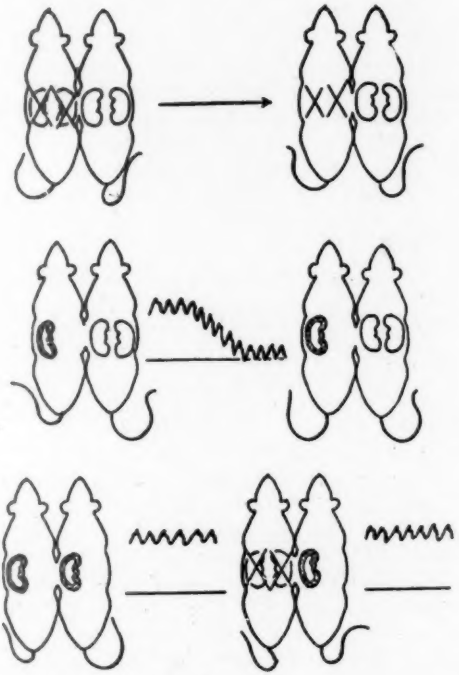

FIG. 9. — *Effect of parabiosis on the blood pressure.* Upper pair: *Parabiosis between a totally nephrectomized rat and a normal rat. The blood pressure of the nephrectomized rat may rise to hypertensive levels; hypertrophy of the kidneys of the normal rat.* Middle pair: *Parabiosis between renal hypertensive rat and normal rat. The blood pressure of the former falls to normal levels; hypertorphy of the kidneys of the normal rat.* Lower pair: *Parabiosis between two hypertensive rats: no fall in blood pressure. Parabiosis between a hypertensive and a totally nephrectomized rat: no fall in blood pressure.*

cardiac output and venous pressure (Grollman, Turner, Levitch and Hill, 1951). Further studies are needed in order to clarify the mechanism of this non renal hypertension, and the possibility of a humoral mechanism should be sought.

b) *Parabiosis.* — A parabiotic union between a nephrectomized rat and a normal rat prolongs the survival of the nephrectomized animal

and causes a marked enlargement of the kidneys of the normal partner (Braun-Menéndez, unpublished data); apparently renotrophin is able to cross the parabiotic barrier.

If hypertension were really due to the inability of the kidney to increase its functional mass, to apportion to the organism of the hypertensive animal a greater amount of normal renal tissue should cause a lowering of the blood pressure. The following experiments of Braun-Menéndez and von Euler (1948) seem to confirm the hypothesis (fig. 9).

When rats with chronic hypertension due to unilateral perinephritis were joined in parabiosis with normal rats the blood pressure of the former fell to normal levels in a few days. Surgical separation of the parabiosed rats was followed by a rapid return of the blood pressure to hypertensive levels. No change in the blood pressure of hypertensive rats followed the parabiotic union of two hypertensive rats or of a hypertensive with a nephrectomized rat (Braun-Menéndez and von Euler, 1948).

General considerations

The hypothesis I have presented and discussed is not without antecedents. Many workers have previously pointed out in differents forms the possibility that the normal kidney "in some manner compensates for the deleterious effects of the ischemia of the other kidney" (Fasciolo, 1938; Katz, Friedman, Rodbard and Weinstein, 1939). Greenwood, Nassim and Taylor (1939), were more explicit and in a paper entitled. "The production of hypertension by the prevention of kidney hypertrophy" they state: "The fact that the normal kidney must be removed in order to cause permanent hypertension suggests either that the normal kidney excretes or destroys the renal pressor substance or that the increased work imposed on the ischemic kidney results in an increased production of the pressor substance. Possibly hypertrophy of the normal kidney reapportions the work so that after some time there is no longer a disproportion between the blood supply and function of the kidney whose artery has been constricted. These two possibilities are not, of course, mutually exclusive". And further on they comment: "It occurred to one of us (N. B. T.) that possibly the release of the renal pressor substance was a physiological response to a disproportion between work required of the kidney and its available blood supply".

More recently Best and Hartroft (1949) have also suggested a disproportion between growth and its demands on the cardiovascular renal system on one hand and kidney damage on the other, as the cause of hypertension in rats fed a choline deficient diet during a short period of time after weanling.

The hypothesis I propose assumes the existence of a substance (or group of substances) which regulates renal growth and function. This substance, wich may be denominated *renotrophin*, is probably connected with the intermediary protein metabolism. Its rate of production increases under the influence of hormones of the anterior hypophysis and the

thyroid and when the animals are fed high protein diets. Conversely it decreases after hypophysectomy, thyroidectomy or the administration of a low protein diet. There is normally an equilibrium between production of renotrophin and its destruction, utilization or transformation by the kidney. An increase of renotrophin production causes hypertrophy, a decrease atrophy of the kidneys. When this equilibrium is ruptured and the remaining renal tissue is unable to respond to the stimulus of the normal or increased amounts of renotrophin present in blood, hypertension develops.

The mechanism of this renal hypertension may be complex: the inability of the kidney to cope with the stimulus may cause it to secrete renin or other vasoactive substance and renotrophin may be itself a pressor substance.

This working hypothesis has some advantages. It is simple since a single substance is appointed the task of regulating renal growth and function, causing renal compensatory hypertrophy and giving rise to hypertension. It does not postulate a mechanism unknown to the organism since we know of many similar interrelations: ACTH regulating adrenal size and function, thyrotrophin regulating thyroid size and function, blood sugar level regulating insulin secretion, etc. And last but not least it offers a plausible explanation of many unto now unexplained phenomena and it opens, I believe, many new avenues for further work.

BIBLIOGRAPHY

ABRAMS, M., SOBIN, S.: *Proc. Soc. exp. Biol.*, N. Y., 1947, *64*, 412.

ADDIS, T.: *Glomerular nephritis. Diagnosis and Treatment;* The Macmillan Co., New York, 1948.

ADDIS, T., LEW, W.: *J. exp. Med.*, 1940, *71*, 325.

ADDIS, T., MYERS, B. A., OLIVER, J.: *Arch. int. Med.*, 1924, *34*, 243.

ADOLPH, E. F.: *Science*, 1949, *109*, 579.

ALLEN, F. M., SCHARF, R., LUNDIN, H.: *J. Amer. med. Ass.*, 1925, *85*, 1698.

ALPERT, L. K., THOMAS, C. B.: *Bull. Johns Hopkins Hosp.*, 1943, *72*, 274.

ANDERSON, E., PAGE, E. W., LI, C. H., OGDEN, E.: *Amer. J. Physiol.*, 1944, *141*, 393.

ANDERSON, H.: *Arch. int. Med.*, 1926, *37*, 313.

APPELROT, S.: *Amer. J. Physiol.*, 1933, *105*, 294.

ARATAKI, M.: *Amer. J. Anat.*, 1926, *36*, 437.

ARNOTT, W. M., KELLAR, R. J.: *Brit. J. exp. Path.*, 1935, *16*, 265.

ARNOTT, W. M., KELLAR, R. J., MATTHEW, G. D.: *Edinburgh med. J.*, 1936, *43*, 233.

BARCROFT, J., BRODIE, T. G.: *J. Physiol.*, 1905, *33*, 52.

BECKMANN, K.: *Deutsches Arch. f. klin. Med.*, 1925, *149*, 177.

BELL, E. T., PEDERSEN, A. H.: *Ann. int. Med.* 1930, *4*, 227.

BEST, C. H., HARTROFT, W. S.: *Fed. Proc.*, 1949, *8*, 610.

BLALOCK, A., LEVY, S. E.: *Ann. Surg.*, 1937, *106*, 826.

BORSOOK, H., DUBNOFF, J. W.: *J. biol. Chem.*, 1940, *134*, 635.

BORSOOK, H., DUBNOFF, J. W.: *J. biol. Chem.*, 1941, *138*, 389.

BRADLEY, S. E., HALPERIN, M. H.: *J. clin. Invest.*, 1948, *27*, 635.

BRAUN-MENÉNDEZ, E.: *Rev. Soc. argent. Biol.*, 1932, *8*, 561; *C. R. Soc. Biol.*, París, 1933, *113*, 461.

BRAUN-MENÉNDEZ, E.: *Rev. Soc. argent. Biol.*, 1946 (a), *22*, 279.

BRAUN-MENÉNDEZ, E.: *Rev. Soc. argent. Biol.*, 1946 (b), *22*, 299.

BRAUN-MENÉNDEZ, E.: *Rev. Soc. argent. Biol.*, 1950, *26*, 65; *C. R. Soc. Biol.*, París, 1950, *144*, 1228.

BRAUN-MENÉNDEZ, E.: Blood volume and volume of extracellular fluid in experimental hypertension; in: *Hipertension - A symposium*, ed. by E. T. Bell, Univ. of Minnesota Press, 1951, page 98.

BRAUN-MENÉNDEZ, E.: The mechanism of hypertension due to desoxycorticosterone; in: *Hypertension - A symposium*, ed. by E. T. Bell, Univ. of Minnesota Press, 1951, page 133.

BRAUN-MENÉNDEZ, E., CHIODI, H.: *Rev. Soc. argent. Biol.*, 1946, *22*, 314.

BRAUN-MENÉNDEZ, E., CHIODI, H.: *Rev. Soc. argent. Biol.*, 1947, *23*, 21.

BRAUN-MENÉNDEZ, E., COVIÁN, M. R.: *Rev. Soc. argent. Biol.*, 1948, *24*, 130; *C. R. Soc. Biol.*, París, 1948, *142*, 1569.

BRAUN-MENÉNDEZ, E., EULER, U. S. VON: *Nature*, 1947, *160*, 905.

BRAUN-MENÉNDEZ, E., EULER, U. S. VON: *Rev. Soc. argent. Biol.*, 1948, *24*, 357.

BRAUN-MENÉNDEZ, E., FASCIOLO, J. C., LELOIR, L. F., MUÑOZ, J. M., TAQUINI, A. C.: *Hipertensión arterial nefrógena;* Buenos Aires, El Ateneo, 1943.

BRAUN-MENÉNDEZ, E., FASCIOLO, J. C., LELOIR, L. F., MUÑOZ, J. M., TAQUINI, A. C.: *Renal Hypertension* - translated by L. Dexter, Charles C. Thomas, Springfield, Ill., 1946.

BRAUN-MENÉNDEZ, E., FOGLIA, V. G.: *Rev. Soc. argent. Biol.*, 1944, *20*, 556.

BRAUN-MENÉNDEZ, E., HOUSSAY, H. E. J.: *Rev. Soc. argent. Biol.*, 1949, *25*, 55.

BRAUN-MENÉNDEZ, E., MARTÍNEZ, C.: *Rev. Soc. argent. Biol.*, 1949, *25*, 162.

BRAUN-MENÉNDEZ, E., PRADO, J. L.: *Rev. Soc. argent. Biol.*, 1950, *26*, 188.

BRAUN-MENÉNDEZ, E., RUÍZ-GUIÑAZÚ, A.: (unpublished data).

BRISKIN, H. L., STOKES, F. R., REED, C. I., MRAZEK, R. G.: *Amer. J. Physiol.*, 1943, *138*, 385.

BRODY, S.: *Bioenergetics and growth;* Reinhold Pub. Corp., New York, 1945.

BROZEK, J., CHAPMAN, C. B., KEYS, A.: *J. Amer. med. Ass.*, 1948, *137*, 1569.

CASH, J. R.: *Bull. Johns Hopkins Hosp.*, 1924, *35*, 168.

CASH, J. R.: *Proc. Soc. exp. Biol.*, N. Y., 1926, *23*, 609.

CHANUTIN, A.: *Arch. int. Med.*, 1934, *54*, 720.

CHANUTIN, A., BARKSDALE, E. E.: *Arch. int. Med.*, 1933, *52*, 739.

CHANUTIN, A., FERRIS, E. B.: *Arch. int. Med.*, 1932, *49*, 767.

CHANUTIN, A., LUDEWIG, S.: *Arch. int. Med.*, 1936 (a), *57*, 887; 1936 (b), *58*, 60; 1936 (c), *58*, 95.

CHANUTIN, A., LUDEWIG, S.: *Arch. int. Med.*, 1939 (a), *64*, 513; 1939 (b), *64*, 526; 1939 (c), *64*, 747.

CLARK, J. K., BARKER, H. G.: *J. clin. Invest.*, 1951, *30*, 745.

COLONGE, R.: *C. R. Soc. Biol.*, París, 1944, *138*, 494.

CROMARTIE, W. J.: *Amer. J. med. Sci.*, 1943, *66*, 206.

DELL'ORO, R.: *Rev. Soc. argent. Biol.*, 1942 (a), *18*, 13.

DELL'ORO, R.: *Rev. Med. and Aliment.*, 1942 (b), *5*, 126.

DE ROBERTIS, E., POCH, G. F.: *Rev. Soc. argent. Biol.*, 1947, *23*, 34.

DÍAZ, J. T., LEVY, S. E.: *Amer. J. Physiol.*, 1939, *125*, 586.

DICK, G. F.: *Arch. Path.*, 1945, *39*, 81.

DICKER, E.: *C. R. Soc. Biol.*, París, 1935, *120*, 793.

DICKER, E.: *Acta med. scand.*, 1937 (a), *92*, 461.

DICKER, E.: *Acta med. scand.*, 1937 (b), *93*, 265.

DICKER, E.: *C. R. Soc. Biol.*, París, 1937 (c), *126*, 910.

DICKER, E.: *Science*, 1948, *108*, 12.

DICKER, S. E., HELLER, H., HEWER, T. F.: *Brit. J. exp. Path.*, 1946, *27*, 158.

DOCK, W.: *Amer. J. Physiol.*, 1933, *106*, 745.

DOMÍNGUEZ, R.: *Arch. Path.*, 1928, *5*, 577.

Amer. J. med. Sci., 1948, *215*, 442. DONTIGNY, P., HAY, E. C., PRADO, J. L., SELYE, H.:

DRURY, D. R.: *J. exp. Med.*, 1938, *68*, 693.

EARLE, D. R., DE BODO, R. C., SCHWARTZ, I. L., FARBER, S. H., KURTZ, M., GREENBERG, J.: *Proc. Soc. exp. Biol., N. Y.*, 1951, *76*, 608.

EILER, J. J., ALTHAUSEN, T. L., STOCKHOLM, M.: *Amer. J. Physiol.*, 1944,, *140*, 699.

FASCIOLO, J. C.: *Rev. Soc. argent. Biol.*, 1938, *14*, 15; *C. R. Soc. Biol., París*, 1938, *128*, 1129.

FASCIOLO, J. C.: *Hipertensión arterial nefrógena. Estudio experimental;* Tesis Doct. Med. Bs. Aires, Ferrari Hnos., 1939.
FERRIS, H. W., HYNES, J. F.: *J. Lab. clin. Med.*, 1931, *16*, 597.
FEYEL, P.: *Ann. d'Endocrinol.*, 1943, *4*, 93.
FOGLIA, V. G., GÜNTHER, B., PINTO, R. M.: *Rev. Soc. argent. Biol.*, 1943, *19*, 384.
FOGLIA, V. G., HOUSSAY, H. E. J.: *Rev. Soc. argent. Biol.*, 1942, *18*, 376.
FONTAINE, T.: *Arch. Sci. phisiol.*, 1947, *1*, 357.
FONTAINE, T., VEIL, C.: *C. R. Soc. Biol.*, París, 1945, *140*, 187.
FONTAINE, T., VEIL, C.: *Arch. Sci. physiol.*, 1947, *1*, 49.
FRIEDBERG, L.: *Amer. J. Physiol.*, 1941, *133*, P. 282.
FRIEDBERG, L.: *Amer. Heart J.*, 1944, *28*, 786.
FRIEDMAN, B., JARMAN, J., KLEMPERER, P.: *Amer. J. med. Sci.*, 1941, *202*, 20.
FRIEDMAN, M., KATZ, L. N.: *J. exp. Med.*, 1938, *68*, 485.
FRIEDMAN, S. M., FRIEDMAN, C. L., POLLEY, J. R.: *Amer. J. Physiol.*, 1948, *153*, 226.
FRIEDMANN, L., WACHSMUTH, W.: *Arch. f. exper. Path. u. Pharmakol.*, 1930, *150*, 173.
GAARENSTROM, J. H.: *Acta brev. neerl. Physiol. Pharmacol. Microbiol.*, 1942, *12*, 107.
GAUDINO, N. M.: *La suprarrenal y el riñón en la hipertensión arterial nefrógena;* Tesis Doct. Med. Bs. Aires, 1944.
GAUDINO, N. M.: *Rev. Soc. argent. Biol.*, 1944, *20*, 470.
GERBI, C.: *Arch. Biochem.*, 1951, *31*, 49.
GLASER, H., LASZLO, D., SCHÜRMEYER, A.: *Klin. Wchnschr.*, 1933, *12*, 138.
GLENN, F., LASHER, E. P.: *Proc. Soc. exp. Biol.*, N. Y., 1938, *38*, 158.
GOLDBLATT, H.: *Ann. int. Med.*, 1937, *11* 69.
GOLDBLATT, H.: *Harvey Lectures*, 1938, 237.
GOLDBLATT, H.: *Amer. J. clin. Path.*, 1940, *10*, 40.
GOLDBLATT, H., BRADEN, S., KAHN, J. R., HOYT, W. A.: *J. Mt. Sinai Hosp.*, 1942, *8*, 579.
GOLDBLATT, H., KAHN, J. R.: *Studies on experimental hypertension. XIII: Experimental observations on the malignant phase of essential hypertension. The production of intrarenal and extrarenal arteriolar necrosis and necrotizing arteriolitis; Blood, Heart and Circulation*, The Science Press, Washington, 1940, page 266.
GOLDBLATT, H., KAHN, J. R.: *J. Amer. med. Ass.*, 1941, *116*, 2428.
GOLDBLATT, H., LYNCH, J., HANZAL, R. F., SUMMERVILLE, W. W.: *J. exp. Med.*, 1934, *59*, 347.
GÓMEZ-SALAZAR, J.: *Hipertensión arterial experimental en la rata;* Tesis Doct. Med. Univ. Católica, Chile, 1942.
GONZÁLEZ, R.: *Rev. Soc. argent. Biol.*, 1938, *14*, 173.
GREENWOOD, W. F., NASSIM, R., TAYLOR, N. B.: *Canad. med. Ass. J.*, 1939, *41*, 443.
GRIFFITH, J. Q. (JR.), INGLE, D. J.: *Proc. Soc. exp. Biol.*, N. Y., 1940, *44*, 538.
GRIFFITH, J. Q. (JR.), LINDAUER, M. A.: *Amer. Heart J.*, 1944, *28*, 295.
GROLLMAN, A.: *Proc. Soc. exp. Biol.*, N. Y., 1944, *57*, 102.
GROLLMAN, A., HALPERT, B.: *Proc. Soc. exp. Biol.*, N. Y., 1949, *71*, 394.
GROLLMAN, A., MUIRHEAD, E. E., VANATTA, J.: *Amer. J. Physiol.*, 1949, *157*, 21.
GROLLMAN, A., TURNER, L. B., LEVITCH, M., HILL, D.: *Amer. J. Physiol.*, 1951, *165*, 167.
GROLLMAN, A., TURNER, L. B., MACLEAN, J. A.: *Arch. int. Med.*, 1951, *87*, 379.
GROLLMAN, A., WILLIAMS, J. R. (JR.): *Amer. J. med. Sci.*, 1942, *204*, 73.
GUERRANT, J. L., SCOTT, J. K., WOOD, J. E. (JR.): *Amer. Heart J.*, 1943, *26*, 232.
HAM, A. W.: *Arch Path.*, 1940, *29*, 731.
HAMORI, A., KORANYI, A.: *Ztschr. f. klin. Med.*, 1938, *133*, 722.
HANDLER, P., BERNHEIM, F.: *Amer. J. Physiol.*, 1950 (a), *160*, 31; 1950 (b), *162*, 189; 1950 (c), *162*, 368; 1950 (d), *162*, 375.
HANDLER, P., BERNHEIM, F.: *Proc. Soc. exp. Biol.*, N. Y., 1951, *76*, 338.
HANDLEY, C. A., MOYER, J. H., KENNEDY, O., COSTA, P.: *J. Pharmacol.*, 1951, *101*, 283.
HANDOVSKY, H. S.: *J. Physiol.*, 1937, *90*, P. 62.
HANTSCHMANN, L.: *Ztschr. f. d. ges. exper. Med.*, 1931, *79*, 1.
HARE, K., PHILLIPS, D. M., BRADSHAW, J., CHAMBERS, G., HARE, R. S.: *Amer. J Physiol.*, 1944, *141*, 187.
HARRISON, T. R., GROLLMAN, A., WILLIAMS, J. R. (JR.): *Amer. J. Physiol.*, 1940, *128*, 716.

HARRISON, T. R., MASON, M. F., RESNIK, H., RAINEY, J.: *Tr. A. Am. Physicians*, 1936, *51*, 280.
HARTMAN, F. W., BOLLIGER, A., DOUB, H. P.: *Amer. J. med. Sci.*, 1929, *172*, 487.
HARTROFT, W. S.: *Brit. J. exp. Path.*, 1948, *29*, 483.
HARTWICH, A.: *Ztschr. f. d. ges. exper. Med.*, 1930, *69*, 462.
HARTWICH, A., HESSEL, G.: *Zentrabl. f. inn. Med.*, 1932, *53*, 612.
HAY, E. C.: *J. Pharmacol.*, 1946, *88*, 208.
HAY, E., SEGUIN, P., LARIVIERE, M., JENSEN, H.: *Fed. Proc.*, 1946, *5*, 137.
HEINBECKER, P., ROLF, D., WHITE, H. L.: *Amer. J. Physiol.*, 1943, *139*, 543.
HERLANT, M.: *Ann. d'Endocrinol.*, 1949, *10*, 313.
HERMANN, H., JOURDAN, F., VIAL, J.: *C. R. Soc. Biol.*, París, 1940, *133*, 523.
HERRMANN, G., DECHERD, G., ERHARD, P.: *Proc. Soc. exp. Biol.*, N. Y., 1941, *47*, 464.
ISRAEL, R.: *These Doct. Med. Fac. Med.*, París, 1938.
JACKSON, C. M., SHIELS, M.: *Anat. Rec.*, 1927, *36*, 221.
JACKSON, H., RIGGS, M.: *J. biol. Chem.*, 1926, *67*, 101.
JANEWAY, T. C.: *Proc. Soc. exp. Biol.*, N. Y., 1908, *6*, 109.
JANEWAY, T. C.: *Amer. J. med. Sci.*, 1913, *145*, 625.
JENSEN, H., CORWIN, W. C., TOLKSDORF, S., CASEY, J. J., BAMMAN, F.: *J. Pharmacol.*, 1941, *73*, 38.
JOLIFFE, N., SMITH, H. W.: *Amer. J. Physiol.*, 1931, *99*, 101.
KATZ, L. N., FRIEDMAN, M., RODBARD, S., WEINSTEIN, W.: *Amer. Heart J.*, 1939, *17*, 334.
KATZ, L. N., MENDLOWITZ, M., FRIEDMAN, M.: *Proc. Soc. exp. Biol.*, N. Y., 1938, *37*, 722.
KATZENSTEIN, M.: *Virchows Arch. f. path. Anat.*, 1905, *182*, 327.
KAY, C. F.: *Arch. Path.*, 1940 (a), *29*, 57.
KAY, C. F.: *J. exp. Med.*, 1940 (b), *72*, 559.
KAY, C. F.: *Am. J. med. Sci.*, 1942, *204*, 483.
KAY, C. F., LUCCHESI, P. F., RUTHERFORD, R. B.: *J. Immunol.*, 1941, *42*, 369.
KEMPF, G. F., PAGE, I. H.: *J. Lab. clin. Med.*, 1942, *27*, 1192.
KNOWLTON, A. I., LOEB, E. N., SEEGAL, B. C., STOERK, H. C.: *Endocrinol.*, 1949, *45*, 435.
KNOWLTON, A. I., LOEB, E. N., STOERK, H., SEEGAL, B. C.: *J. exp. Med.*, 1947, *85*, 187.
KNOWLTON, A. I., STOERK, H., SEEGAL, B. C., LOEB, E. N.: *Endocrinol.*, 1946, *83*, 315.
KOCHAKIAN, C. D., STETTNER, C. E.: *Amer. J. Physiol.*, 1948, *155*, 255.
KONZETT, H., UNNA, K.: *Arch. exp. Path. Pharmakol.*, 1937, *186*, 694.
KORENCHEWSKY, V., HALL, K., BURBANK, R.: *Biochem. J.*, 1939, *33*, 372..
LEATHEM, J. H.: *Proc. Soc. exp. Biol.*, N. Y., 1947, *64*, 90.
LEATHEM, J. H.: *Amer. J. Physiol.*, 1948, *154*, 459.
LOEB, E. N., KNOWLTON, A. I., STOERK, H. C.: SEEGAL, B. C.: *J. exp. Med.*, 1949, *89*, 287.
LOOMIS, D.: *Arch. Path.*, 1946, *41*, 231.
LÖWENTHAL, K.: *Klin. Wchnschr.*, 1926, *5*, 478.
LUDDEN, J. B., KRUEGER, E., WRIGHT, I. S.: *Endocrinol.*, 1941, *28*, 619.
MACKAY, E. M., MACKAY, L. L.: *J. Nutrition*, 1931, *4*, 33.
MACKAY, E. M., MACKAY, L. L., ADDIS, T.: *Amer. J. Physiol.*, 1928, *86*, 459.
MACKAY, E. M., MACKAY, L. L., ADDIS, T.: *Amer. J. Physiol.*, 1928, *86*, 466.
MACKAY, E. M., SMITH, F. M., CLOSS, K.: *J. Nutrition*, 1933, *6*, 151.
MACKAY, L. L., MACKAY, E. M., ADDIS, T.: *J. clin. Invest.*, 1925, *1*, 576.
MACLACHLAN, I., TAYLOR, N. B.: *Amer. J. Physiol.*, 1940, *129*, P. 413.
MAEGRAITH, B. G., MCLEAN, F. J.: *J. Physiol.*, 1938, *92*, P. 44.
MAGALDI, J. B.: *Hipertensão arterial experimental do rato;* Tesis Doct. med. Univ. Sao Paulo, 1945.
MARK, R. E.: *Ztschr. ges. exper. Med.*, 1925, *46*, 1.
MARK, R. E.: *Ztschr. ges. exper. Med.*, 1928, *59*. 601.
MARK, R. E., GIESENDÖRFER, H.: *Ztschr. ges. exper. Med.*, 1930, *74*, 350.
MASSON, G., CORCORAN, A. C., PAGE, I. H.: *J. Lab. clin. Med.*, 1949, *34*, 1416.
MASUGI, M.: *Beitr. path. Anat. allg. Path.*, 1934, *92*, 429.
MCQUEEN-WILLIAMS, M., THOMPSON, K. W.: *Yale J. Biol. Med.*, 1940, *12*, 531.

MEGIBOW, R. S., FRIEDBERG, L., RODBARD, S., KATZ, L. N.: *Proc. Soc. exp. Biol.*, N. Y., 1940, *43*, 245.
MEGIBOW, R. S., KATZ, L. N.: *Amer. J. Physiol.*, 1941, *133*, P. 386.
MEGIBOW, R. S., KATZ, L. N., RODBARD, S.: *Amer. J. med. Sci.*, 1942, *204*, 340.
MILLER, A. J.: *J. exp. Med.*, 1925, *42*, 897.
MOISE, T., SMITH, A. H.: *Proc. Soc. exp. Biol.*, N. Y., 1926, *23*, 561.
NEWBURGH, L. H.: *Arch. int. Med.*, 1919, *24*, 359.
NEWBURGH, L. H., CLARKSON, S.: *Arch. int. Med.*, 1923, *32*, 850.
NUZUM, F. R., OSBORNE, M., SANSUM, W. D.: *Arch. int. Med.*, 1925, *35*, 492.
NUZUM, F. R., SEEGAL, K., GARLAND, K., OSBORNE, M.: *Arch. int. Med.*, 1926, *37*, 733.
OSBORNE, T. B., MENDEL, L. B.: *J. biol. Chem.*, 1924, *59*, 13.
PAGE, E. W., PATTON, H. S., OGDEN, E.: *Amer. J. Obst. Gynec.*, 1941, *41*, 53.
PAGE, I. H.: *Amer. J. med. Sci.*, 1936, *191*, 251.
PAGE, I. H.: *J. Amer. med. Ass.*, 1939 (a), *113*, 2046.
PAGE, I. H.: *Science*, 1939 (b), *89*, 273.
PAGE, I. H., LEWIS, L. A.: *Amer. J. Physiol.*, 1949, *156*, 422.
PAGE, I. H., SWEET, J. E.: *Amer. J. Physiol.*, 1937, *120*, 238.
PÄSSLER, H., HEINECKE, D.: *Verhandl. deutsch. Ges. inn. Med.*, 1904, *9*, 99.
PATTON, H. S., PAGE, E. W., OGDEN, E.: *Surg. Gynec. Obstet.*, 1943, *76*, 493.
PEDERSEN, A. H.: *Arch. Path.*, 1927, *3*, 912.
PETERS, J. P., VAN SLYKE, D. D.: *Quantitative clinical chemistry. Interpretations.* Vol. I, The Williams and Wilikins Co., Baltimore, 2nd Ed., 1946.
PHILIPSBORN, H., KATZ, L. N., RODBARD, S.: *J. exp. Med.*, 1941, *74*, 591.
PICKERING, G. W., PRINZMETAL, M.: *Clin. Sci.*, 1938, *3*, 357.
PRADO, J. L., DONTIGNY, P., HAY, E. C., SELYE, H.: *Fed. Proc.*, 1947, *6*, 182.
PRADO, J. L., DONTIGNY, P., HAY, E. C., SELYE, H.: *Proc. Soc. exp. Biol.*, N. Y., 1947, *66*, 446.
PULLMAN, T. N., ALVING, A. S., LANDOWNE, M.: *Fed. Proc.*, 1949, *8*, 129.
RAPOPORT, S., BRODSKY, W. A., WEST, C. D.: *Amer. J. Physiol.*, 1949, *157*, 357.
RATHER, L. J.: *J. exp. Med.*, 1950, *92*, 59.
RAU, G. C.: *Science*, 1950, *111*, 229.
RAUTENBERG, E.: *Deutsche med. Wchnschr.*, 1910, *36*, 551.
READER, V. B., DRUMMOND, J. C.: *J. Physiol.*, 1925, *59*, 472.
REID, C.: *J. Physiol.*, 1944, *103*, P. 12.
REID, C.: *J. Physiol.*, 1947, *106*, P. 19.
RHOADS, C. P., ALVING, A. S., HILLER, A., VAN SLYKE, D. D.: *Amer. J. Physiol.*, 1934, *109*, 329.
RICHARDSON, J. A., HOUCK, C. R.: *Amer. J. Physiol.*, 1951, *165*, 93.
ROBBERS, H., WESTENKOEFFER, O.: *Ztschr. ges. exper. Med.*, 1939, *105*, 180.
RODBARD, S., FREED, S. C.: *Endocrinol.*, 1942, *30*, 365.
ROLLASON, H. D.: *Anat. Rec.*, 1946, *94*, 492.
ROLLASON, H. D.: *Anat. Rec.*, 1949, *104*, 263.
RYTAND, D. A., DOCK, W.: *Arch. int. Med.*, 1935, *56*, 511.
SCHÖNHEIMER, R.: *Virchows Arch. path. Anat.*, 1924, *249*, 1.
SCHROEDER, H. A.: *J. exp. Med.*, 1942, *75*, 513.
SELYE, H.: *J. Urol.*, 1939, *42*, 637.
SELYE, H.: *J. Urol.*, 1941, *46*, 110.
SELYE, H.: *Canad. med. Ass. J.*, 1942 (a), *47*, 515.
SELYE, H.: *Rev. canad. Biol.*, 1942 (b), *1*, 577.
SELYE, H.: *J. Amer. vet. med. Ass.*, 1943, *103*, 140.
SELYE, H.: *Meet. Roy. Soc. Canada*, Montreal, May, 1944.
SELYE, H.: *Canad. med. Ass. J.*, 1944, *50*, 426.
SELYE, H.: *J. clin. Endocrinol.*, 1946, *6*, 117.
SELYE, H.: *Proc. Inst. Med. Chicago*, 1948, *17*, N° 2.
SELYE, H.: *The physiology and pathology of exposure to stress. A treatise based on the concepts of the general adaptation syndrome and the diseases of adaptation.* Montreal, Acta Inc. Med. Publishers, 1950.
SELYE, H.: *Brit. med. J.*, 1951 (a), *I*, 263.
SELYE, H.: *Lancet*, 1951 (b), *I*, 483.
SELYE, H.: *Rev. canad. Biol.*, 1951 (c), *9*, 475.

SELYE, H., HALL, C. E.: *Arch. Path.*, 1943, *36*, 19.
SELYE, H., HALL, C. E.: *Amer. Heart J.*, 1944, *27*, 338.
SELYE, H., HALL, C. E., ROWLEY, E. M.: *Canad. med. Ass. J.*, 1943, *49*, 88.
SELYE, H., MARTÍNEZ, H., THOMSON, D. L., COLLIP, J. B.: *Arch. Path.*, 1934, *18*, 878.
SELYE, H., PENTZ, E. I.: *Canad. med. Ass. J.*, 1943, *49*, 264.
SELYE, H., ROWLEY, E. M.: *Fed. Proc.*, 1944, *3*, 41.
SELYE, H., STONE, H.: *Proc. Soc. exp. Biol.*, N. Y., 1943, *52*, 190.
SELYE, H., STONE, H., NIELSEN, K., LEBLOND, C. P.: *Canad. med. Ass. J.*, 1945, *52*, 571.
SELYE, H., STONE, H., TIMIRAS, P. S., SCHAFFENBURG, C.: *Amer. Heart J.*, 1949, *37*, 1009.
SMADEL, J. E.: *J. exp. Med.*, 1937, *65*, 541.
SMADEL, J. E., FARR, L. E.: *J. exp. Med.*, 1937, *65*, 527.
SMITH, A. H., MOISE, T. S.: *J. exp. Med.*, 1927, *47*, 263.
SMITH, H. W.: *The physiology of the kidney.* Oxford Med. Publicat., London, 1937.
SMITH, P. E.: *Amer. J. Anat.*, 1930, *45*, 205.
SOBIN, S. S., LANDIS, E. M.: *Amer. J. Physiol.*, 1947, *148*, 557.
SULKIN, N. M.: *Anat. Rec.*, 1949, *105*, 95.
SULKIN, N. M.: *Anat. Rec.*, 1950, *106*, 290.
SWANN, H. G.: *Proc. Soc. exp. Biol.*, N. Y., 1939, *40*, 520.
VAN SLYKE, D. D., PHYLLIPS, R. A., HAMILTON, P. B., ARCHIBALD, R. M., FUCHTER, P. H., HILLER, A.: *J. biol. Chem.*, 1943, *150*, 481.
VAN SLYKE, D. D., RHOADS, C. P., HILLER, A., ALVING, A. S.: *Amer. J. Physiol.*, 1934, *109*, 336.
VERNEY, E. B., VOGT, M.: *Quart. J. exp. Physiol.*, 1938, *28*, 253.
VERNEY, E. B., VOGT, M.: *Quart. J. exp. Physiol.*, 1943, *32*, 35.
WALTER, F., ADDIS, T.: *J. exp. Med.*, 1939, *69*, 467.
WATSON, C. W., LYON, G.: *J. Physiol.*, 1906, *34*, P. 19.
WELSH, C. A., ROSENTHAL, A., DUNCAN, M. T., TAYLOR, H. C.: *Amer. J. Physiol.*, 1942, *137*, 338.
WHITE, H. L., HEINBECKER, P.: *Amer. J. Physiol.*, 1940, *130*, 464.
WHITE, H. L., HEINBECKER, P., ROLF, D.: *Proc. Soc. exp. Biol.*, N. Y., 1941, *46*, 44.
WHITE, H. L., HEINBECKER, P., ROLF, D.: *Amer. J. Physiol.*, 1942, *136*, 584.
WHITE, H. L., HEINBECKER, P., ROLF, D.: *Amer. J. Physiol.*, 1947, *149*, 404.
WHITE, H. L., HEINBECKER, P., ROLF, D.: *Amer. J. Physiol.*, 1949, *157*, 47.
WHITE, H. L., HEINBECKER, P., ROLF, D.: *Amer. J. Physiol.*, 1951, *165*, 442.
WILSON, C., BYROM, F. B.: *Quart. J. Med.*, 1941, *10*, 65.
WINTERNITZ, M. C. MYLON, E., WATERS, L. L., KATZENSTEIN, R.: *Yale J. Biol. Med.*, 1940, *12*, 623.
WINTERNITZ, M. C., WATERS, L. L.: *Yale J. Biol. Med.*, 1940, *12*, 705.
WOLF, H. J., HEINSEN, H. A.: *Arch. exper. Path. Pharmakol.*, 1935, *179*, 15.
WOOD, J. E. (JR.), CASH, J. R.: *Ann. int. Med.*, 1939, *13*, 81.
WOOD, J. E. (JR.), ETHRIDGE, C.: *Proc. Soc. exp. Biol.*, N. Y., 1933, *30*, 1039.
ZECKWER, I. T.: *Amer. J. Physiol.*, 1946, *145*, 681.
ZUCKER, M. B., CIZEK, L. J., TOMPKINS, D.: *Amer. J. Physiol.*, 1950, *162*, 162.

INHIBITORY INFLUENCES ON THE SENSITIVITY OF SPINAL CORD NEURONES

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INHIBITORY influences on the chemical sensitivity of spinal cord neurones that are mediated through the dorsal roots have been observed (Drake and Stavraky, 1948). The research reported in the present paper was undertaken to learn whether this inhibitory action might be due to some spontaneous activity of cells of the spinal ganglia. Our results offer the possibility to discuss the question of whether influences here in play are effects depending on nerve cell activity, but not related with nerve impulses.

An interpretation of the determinism of the supersensitivity of denervated structures is also suggested.

METHODS

Non selected adult cats were used through the 30 experiments performed. The animals were operated under aseptic conditions and were anesthetized with ether or Evipal * (0.75 mg per kg, intraperitoneally). Laminectomy was performed at levels from L_7 to S_4. The spinal nerves and ganglia were carefully isolated with glass hooks. Nerve sections were achieved intradurally and with no damage to the *pia mater*. A small piece of nerve was always removed. In some instances the spinal ganglion was removed, in others, the nerve was cut peripherally to the ganglion. In order to avoid the effects of retrograde degeneration of the motor nerves, in many experiments only the sensory branch was severed.

Between 6 to 75 days later, the cat was pithed under a short rush of ether and the experiment started only after the anesthetic was eliminated. The vertebral canal was opened to obtain an ample space which allowed comfortable manipulations. The animal was fixed to the table with drills inserted into both sides of the pelvic bones. The dorsal and

* Barbitutate compound, generously provided by Winthrop Stearn, Inc.

ventral roots corresponding to the operated nerve and those of the contralateral side were isolated. By means of steel pins and rubber bands a cup was formed with the skin and filled with mineral oil. The preparation was maintained at about 37.5°C by radiant heat from an infrared bulb. Monotopic action potentials from the ventral roots were recorded. The electrodes were fine platinum wires mounted on ball and socked joints attached to calibrated micrometer screws. This arrangement allowed displacements in almost any direction and very fine adjustments. The action potentials were led to a Grass pre-amplifier which fed a Cossor double-beam oscillograph; they were photographed with a Grass kymograph cammera. The film or the paper speed was always 10 mm

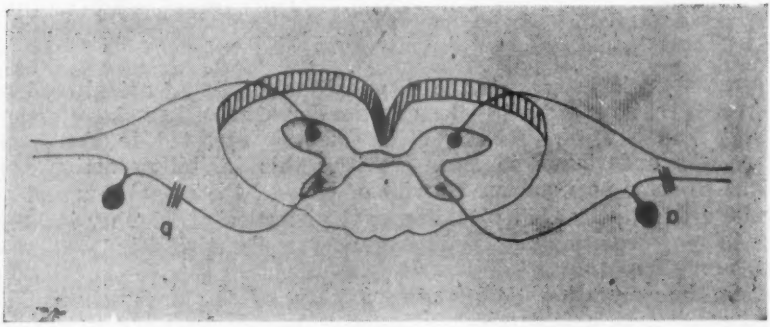

FIGURE 1. — *Diagram illustrating the site of the nerve sections described in the text.* a) *peripherally to the ganglion;* b) *centrally to the ganglion (dorsal root).*

per second. Both sides were simultaneously recorded. The preparation was maintained deeply curarized with raw curare from Venezuela. In a few experiments d-tubocurarine was used. The depth of curarization was tested by stimulating the sciatic nerve with a Harvard inductorium. Spinal cord stimulation was obtained by partial asphyxia (cutting off the artificial respiration) or by injecting intravenously strychnine (about 0.25 mg per kg) or metrazol (about 30 mg per kg).

RESULTS

I. — *Effects due to acute section of dorsal roots.*

In a fully curarized spinal cat the chemical sensitivity of the spinal cord nerve cells was studied by recording nerve discharges through one ventral root (usually S_1 or S_2), before and after the severance of the corresponding dorsal root (fig. 1, b). The simplest way to stimulate the spinal cord neurones was by partial asphyxia. The intensity of the stimulus was easily controlled. Stimulation could be stopped and repeated at

will. Figure 2 illustrates a typical observation. It is clear that the acute section of the dorsal root did not modify the response. From figure 3, a similar conclusion can be drawn, using strychnine as stimulating agent and recording from both sides.

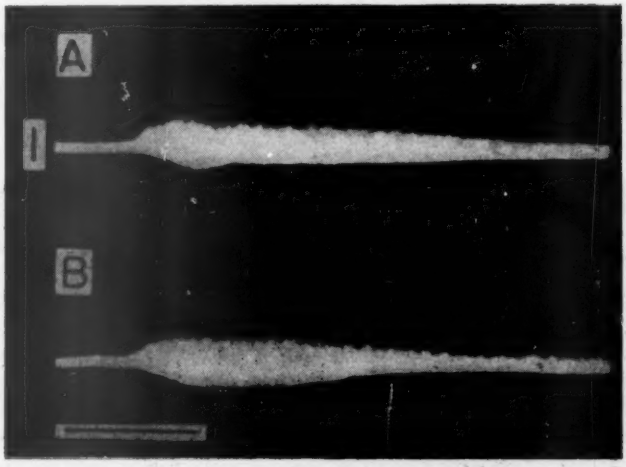

FIGURE 2. — *Effect of acute section of a dorsal root on the discharges of motoneurones during partial asphyxia. Responses were recorded on* S_1 *ventral root.* A: *response obtained with intact dorsal root.* B: *same as A, but after section of the corresponding dorsal root see fig. 1, b). Cal: 100* μV. *Time: 0.5 s.*

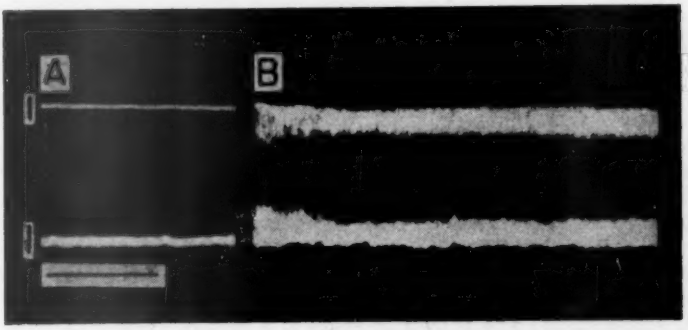

FIGURE 3. — *Effect of acute section of a dorsal root in the discharges of motoneurones to strychnine. Responses were recorded on the left and on the right* S_2 *ventral roots. The corresponding dorsal root (fig. 1, b) was cut shortly before the injection of the drug on the right side. Upper beam: right side. Lower beam: left side. A: recorded immediately after the injection, but before the appearence of any activity. B: a few seconds later, during the outburst of activity. Cal: 200* μV. *Time: 0.5 s.*

II. — *Effects arising from the spinal ganglia neurones.*

To study the effects of the spinal ganglion cells on the chemical sensitivity of the spinal cord nerve cells, the cats were bilaterally operated. At one side (fig. 1, b) one dorsal root was cut (usually the S_1 ganglion or the S_2 ganglion was removed) and at the opposite side (fig. 1, a) the nerve was severed peripherally to the ganglion. The operation was performed from 6 to 75 days prior to the experiment. The section of the motor branch together with the sensory one, might result in a physiological alteration of the motoneurones by retrograde degeneration. Acheson, Lee and Morison (1942) have observed a temporary deficiency of the cut

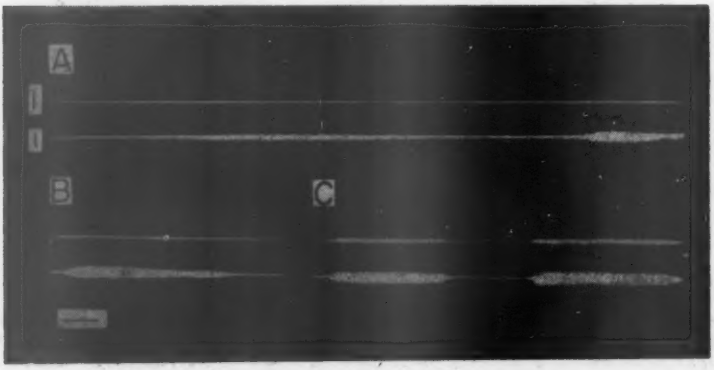

FIGURE 4. — *Influence of the spinal ganglion on the sensitivity of the spinal cord neurones to asphyxia. Discharges were recorded from ventral roots S_1. On the right side (lower beam) the severance shown in figure 1, b, was performed on S_1 spinal ganglion 12 days before. On the left side, severance shown in figure 1, a, was performed at S_1 by the same time.* A: *the first recorded responses during asphyxia.* B: *six seconds later.* C: *seventeen seconds after B. Cal: 200 μV. Time: 1 s.*

phrenic nerve with a time course similar to that of the retrograde degeneration. In any case, the effect of this degeneration would be equal at both sides and by this reason it was controlled in every experiment. But, to avoid any interference with the retrograde degeneration of motoneurones, in 7 out of 16 experiments, only the sensory branch was cut. In both groups the results were quite similar. During the experiment the ganglion, whose peripheral axone had been previously cut, was removed. Typical experiments are shown in figures 4 and 5. The effects were various: in most of the experiments, at the beginning of the asphyxia, discharges were present solely on the side where the glanglion had been chronically removed. Later on, when asphyxia had progressed the reaction was present on both sides, but it was stronger on the previously operated one. During a deeper asphyxia, discharges were practically equal on both sides. Similar results were obtained by stimulating either with

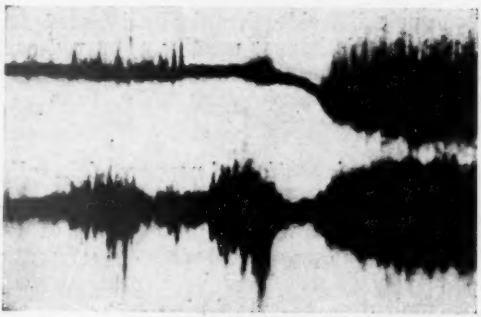

FIGURE 5. — *Influence of the spinal ganglion on the chemical sensitivity of the spinal neurones. On the right side the section was performed —as shown in figure 1, a— 32 days before. On the left side the severance was done, as shown in figure 1, b, by the same time. Both sections were performed at* S_1. *Upper beam: discharges recorded from the corresponding right ventral root. Lower beam: activity recorded from the corresponding left ventral root. A few seconds before the recording, 0.15 mg of strychnine was injected intravenously. Cal: 50* μV. *Time: 0.5 s.*

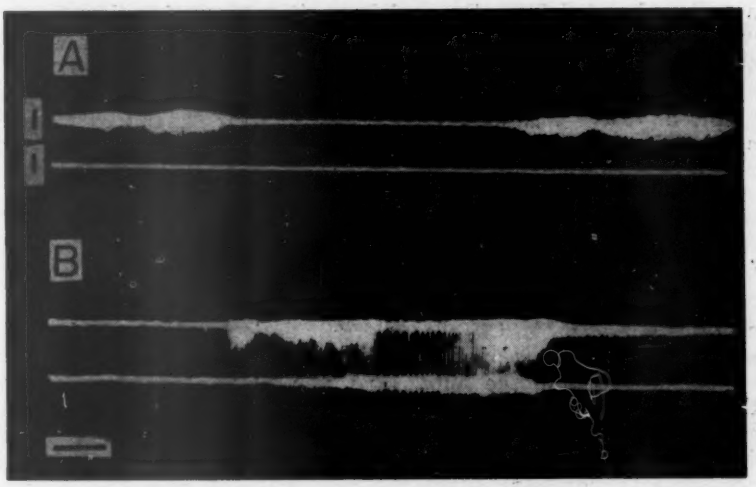

FIGURE 6. — *Effect of chronic section of the sensory spinal nerve, peripherally to the ganglion, on the sensitivity to asphyxia of spinal neurones. The corresponding ganglia of both sides were acutely removed. The nerve of the left side was cut, as shown in figure 1, a, 11 days prior to the experiment. Upper beam: discharges recorded from the ventral root of the chronically denervated side (left side). Lower beam: discharges from the normal side.* A: *responses during asphyxia.* B: *same as A, but between A and B, 0.5 mg of strychnine was injected. Cal: 100* μV. *Time: 1 s.*

strychnine or metrazol: the side chronically isolated from the ganglion was more sensitive. The discharges usually appeared earlier on this side and they lasted longer. The responses were practically equal on both sides when discharges attained its maximal intensity. In other words, the capacity to react had not been disturbed.

III. — *Effects due to chronic section of nerve peripherally to the ganglion.*

Methods were quite similar to those of part II. One side was operated (fig. 1, a), the other side was left intact. But, of course, the ganglia on both sides were removed during the acute experiment. Figure 6 shows the increased sensitivity to asphyxia by chronic nerve section. In this particular case a small dose of strychnine was used to render the responding neurones more sensitive to asphyxia. The potentiation to asphyxia by strychnine was also more pronounced at the operated side.

IV. — *Spontaneous electrical activity of the spinal ganglia.*

It seemed important to know whether nerve impulses generate spontaneously in the cell bodies of the spinal ganglion. Should this be so, we might assume that the inhibitory influence from the ganglia is mediated by nerve impulses. In a group of experiments the recording electrodes were placed on one dorsal root, in order to observe possible spontaneous discharges arising from the ganglion. In some experiments the ganglion was acutely isolated from the periphery (fig. 1, a), in other cases, the nerve was severed several days before. Monotopic recording was used. There was no difference between the two groups of experiments.

The observation was run for periods longer than one hour, and no spontaneous discharges were recorded. In a few experiments asphyxia was induced or strychnine was injected in order to stimulate the ganglion, but no response was observed.

Discussion

Effect of acute deafferentation.

Drake and Stavraky (1948), have shown that immediately after section of the dorsal roots, the chemical stimulation of the spinal cord was less effective for several hours. However, they have neither recorded directly the activity of the spinal cord, nor the motoneurones discharges at the ventral roots. In their experiments, the activity of the spinal cord after chemical stimuli was tested indirectly through the response of skeletal muscles. On the contrary, the method used in this paper procures a more direct knowledge about the sensitivity of spinal neurones and also isolates these neurones from muscular influences, because some motor roots were severed and because a fully curarized animal was used.

Under normal conditions the ventral roots of both sides and at the same level, responded with almost equal intensity to asphyxia and to chemical stimuli. This observation makes the preparation an appropiate one to test the relative excitability of spinal neurones. As it was mentioned before (results, part I), there was neither a decreased nor an increa-

sed response to asphyxia or strychnine shortly after the section of the dorsal roots of one of the spinal nerves. This inference has been demonstrated by comparing the response on the same side before and after acute deafferentation, and by comparing the responses of both sides, one normal and the other with the dorsal root cut (figs. 2 and 3). Thus it becomes evident that the decreased muscular response observed by Drake and Stavraky after acute deafferentation is not due to a change of chemical sensitivity of the spinal cord neurones.

Effects of chronic deafferentation.

The stimuli applied may have activated directly the motoneurones; but they may have influenced also some internuncial neurones which, through one or more synapses, induce the motoneurones of both sides to discharge. It seems reasonable to assume that the action potentials recorded on the ventral roots are due to both, the direct and the indirect activation of the anterior horn cell. However, with the method used here any effect of chronic deafferentation can not be demonstrated in its full intensity, because both sides are partially denervated. But, only at the same side of the deafferentation some of the anterior horn cells are directly denervated. At the opposite side, denervation of the motoneurones is only an indirect one, i. e., some internuncial neurones, that make contact with them through synapses, have been isolated from the ganglion.

According to the analysis given in the foregoing paragraphs, it seems justified to infer that any dissimilarity in behavior of the motoneurones of the side with the dorsal root previously cut and motoneurones of the opposite side is quite significant.

The present study confirms the statement of Drake and Stavraky that chronic severance of the dorsal roots leaves a supersensitivity of the spinal neurones.

As shown in this paper the inhibitory effect depends on two factors: a) an influence from the spinal ganglia and b) an influence coming from the periphery.

a) *Influences arising from the spinal ganglia.* — The different effects obtained after section of the dorsal roots and after section of the spinal sensory nerves, demonstrate that the inhibition is, at least in part, originated in the spinal ganglia cells.

The next question to discuss is the mechanism by which the ganglia cells influence the excitability of the spinal neurones. As it has been described in part IV, no spontaneous electrical discharges could be recorded from the ganglion. Of course, this negative result does not exclude the possibility that the ganglia might discharge spontaneously, but under our present knowledge it is necesary to discuss ganglionic influences as due to non nerve-impulse activity of the neuron.

The lack of electrical activity in an isolated neurone is not a news. Tower (1937), studying partially denervated spinal neurones in dogs, concluded that there was no recorded spontaneous activity in the mammalian spinal cord. Tower, Bodian and Howe (1941) reported a similar

conclusion in experiments carried on in monkeys. Govaerts (1936) claimed to have recorded spontaneous discharges from an isolated sympathetic ganglion. But, as Tower (1939) has adduced it seems necessary to confirm this assertion.

Usually, spontaneous activity of a neurone is understood as a discharge of nerve-impulses. But it is possible to conceive that there is an activity of a nerve cell which is not mediated by nerve-impulses. One may assume that such an activity is a spontaneous one, i. e., that it is generated in the cell, depending on the normal or optimal metabolism of the latter, but not on any stimuli or changes of the "*milieu interne*". The results reported in this paper give evidence that the spinal ganglion does not discharge nerve-impulses, and that nevertheless it depresses the sensitivity of spinal neurones. Therefore, it seems necessary to assume that a neurone may have an effect on a post-neuronic element by some other way than nerve-impulses.

In the experiments of Tower, Howe and Bodian (1941) we find a similar situation. Some spinal neurones were isolated in monkeys; the motoneurones continued innervating skeletal muscles. The attached muscles were indeed paralyzed and underwent atrophy by disuse. Nevertheless, no fibrillation was present. But when the motor nerve was cut the well known fibrillation produced by denervation appeared. It is clear that isolated spinal neurones exercise some action on the attached muscles. This activity which results in an inhibition of muscular fibrillation is characterized by two aspects which are of a special interest in our context: it seems that it is spontaneous, and, on the other hand, it is not mediated by nerve-impulses.

Recently Fatt and Katz (1950) have given another clearcut instance of a post-neuronic effect, depending on neuronic activity in absence of nerve-impulses: the miniature end-plate potential. They interpret this small potential at the end plate as due to a release of small quantities of acetylcholine from motor nerve terminals due to spontaneous fluctuations of their membrane potential. This explanation is admissible if the presynaptic nerve is a cholinergic one. But the case discussed in our paper does not meet this condition.

It has been interesting to find in recent literature what may be called the photographic negative of the spinal ganglion effect reported in this paper. Sidman and Singer (1951) found that the spinal ganglia cells isolated from the central nervous system can stimulate regeneration of the new limb in the newt. They infer that the growth-stimulating action arises in the neurones of the spinal ganglia and that it is probably independent of impulse conduction. In other words, we are dealing in our experiments with an activity arising in the spinal ganglion cells and producing its effect on the central nervous system; on the other hand, Sidman and Singer are dealing with an activity originating in the glanglion, but producing its effect on the periphery. Both influences are probably independent of nerve-impulses.

The chronic and partial isolation of the spinal cord nerve cells from

the spinal ganglion results in the conditions described by the known law of denervation (Cannon and Rosenblueth, 1949): a) the cells become more sensitive to chemical stimuli; b) their reactivity is not altered, i. e., the maximal response is unchanged; c) supersensitivity is greater for the links which immediately follow the cut neurones and less intense for the distant cells. In a recent monograph Cannon and Rosenblueth (1949) discussed the determinism of sensitization of denervated structures. Two factors are especially considerd: the rôle of a total or partial inactivation that follows denervation, and the rôle of the lack of chemical mediators that normally activate the cell. In our experiments, none of these two factors seem to be related with the supersensitivity observed. The activity of the spinal nerve cells of the same side where the glanglion was chronically removed, is probably the same than the activity of the side where only the nerve, distally to the ganglion, was cut (see results, part II). This assumption is based on the fact that no nerve-impulses have been recorded on the dorsal root in a peripherally isolated ganglion (see results, part IV). In regard to the chemical mediators, we have to consider, under the present knowledge, the axone of the spinal ganglion neither as a cholinergic nor as an adrenergic fibre. Conclusively, the present study strongly suggests that the common factor responsible for the supersensitivity after denervation is neither inactivity nor the lack of chemical mediators. It also suggests that this factor is a non nerve-impulse influence.

For the moment, any theory of the nature of the factor in play would be purely speculative. Before trying any explanation, it will be necessary to answer various questions: Is there any substances "leaking" from the nerve endings that might interfere with the post-synaptic element? Does the resting membrane potential change spontaneously at the nerve endings in minor values that might influence the post-synaptic cell? Is the effect we have observed a "trophic" one?

b) *The peripheral influences.* — The unequal response of the spinal neurones of both sides to chemical stimuli, which we have observed when the sensory nerve of one side was cut peripherally to the ganglion, warrants the assumption that the sensitivity of these neurones may also depend on influences coming from the periphery. Nerve impulses are evidently not necessary for this effect since sensitivity is not modified shortly after the section of the dorsal roots. Is this effect on the spinal neurones an independent one, or is it possible to interpret this effect as a modification of the inhibitory activity emanating from the spinal glanglion? The latter assumption is based on the well known fact that the severance of a portion of a neurone produces anatomical and physiological alterations in the remaining cell. The anatomical changes are known as retrograde degeneration. The physiological changes might result in a decrease of glanglionic activity and this would mean a release of the spinal neurones inhibition due to influences arising from the ganglion.

SUMMARY

The present paper deals with experiments which render it probable that inhibitory influences on the sensitivity of the spinal cord neurones may arise from the spinal ganglion cells.

The response of the spinal cord neurones was tested by recording electrical discharges from the motoneurones after partial asphyxia or drug stimulation.

The following experimental statements were made:

1). — Acute elimination of the spinal ganglion did not modify the sensitivity of the spinal cord neurones.

2). — On the contrary, spinal cord neurones, chronically isolated from the spinal ganglion showed to be more sensitive to chemical stimuli that the control corresponding neurones.

3). — Nevertheless, no spontaneous discharges from the isolated spinal ganglion could be recorded on the dorsal roots.

On the basis of these findings, it is suggested that the inhibitory action of the spinal ganglion neurones on the spinal cord nerve cells is due to a non nerve-impulse influence.

• We wish to express our grateful acknowledgement to the Rockefeller Foundation (New York) and to the Gildemeister Foundation (Santiago, Chile) for grants to equip and install this new laboratory.

REFERENCES

ACHESON, G. H., LEE, E. S., MORISON, R. S.: *Neurophysiol.*, 1942, *5*, 269.
CANNON, W. B., ROSENBLUETH, A.: *The supersensitivity of denervated structures*, New York, The Macmillan Company, 1949.
DRAKE, CH. G., STAVRAKY, G. W.: *J. Neurophysiol.*, 1948, *11*, 229.
FATT, P., KATZ, B.: *Nature.*, 1950, *166*, 597.
GOVAERTS, J.: *C. R. Soc. Biol.*, Paris, 1936, *121*, 854.
SIDMAN, R. L., SINGER, M.: *Amer. J. Physiol.*, 1951, *165*, 257.
TOWER, S. S.: *J. Comp. Neurol.*, 1937, *67*, 109.
TOWER, S. S.: *Physiol. Rev.*, 1939, *19*, 1.
TOWER, S. S., BODIAN, D., HOWE, H.: *J. Neurophysiol.*, 1941, *4*, 388.
TOWER, S. S., HOWE, H., BODIAN, D.: *J. Neurophysiol.*, 1941, *4*, 398.

FAILURE OF VITAMIN B_{12} TO REDUCE ALCOHOL INTAKE IN N_1 DEPRIVED RATS

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IN PREVIOUS papers from this Laboratory ([1, 4, 9]) it has been shown that rats voluntarily consume large quantities of alcohol when maintained with a diet deprived of a factor present in liver and yeast. We have also shown that this factor, initially called factor N, is actually composed at least of two factors: one thermolabile identified as thiamin, and another thermostable, different from riboflavin, pantothenate, niacin and pyridoxin. The latter has been provisionally called factor N_1 ([5, 6]).

The results reported by Bray and Westerfeld ([1]) and by ourselves ([7]) show that factor N_1 is also different from inositol, PABA, choline chloride, folic acid and biotin.

The presence of individual differences in the alcohol intake of rats, complicates the interpretation of the results. These individual differences have been observed independently by Williams, Berry and Beerstecher ([10]) and by ourselves ([2]). Notwithstanding these individual fluctuations, it is evident that an important proportion of rats exhibit a high level of voluntary alcohol intake when the diet is lacking factor N_1 and show a drop of this intake when sources of this factor are added. Disregarding some exceptions, it is justifiable to state that the thermostable vitamins already mentioned are not capable of diminishing the alcohol intake of N_1 deprived rats.

This paper deals with the effect of vitamin B_{12} on the alcohol intake of rats, maintained with a diet containing a sufficient amount of the ten mentioned vitamins. Nearly two years ago, we have tested it in daily doses of 0.23 μg and 0.46 μg given subcutaneously in small groups of rats. Recently we have been able to study its effect in bigger doses and in a greater number of rats *. Now we have given it by mouth in doses of 10 μg every other day. Both experimental series are considered in this paper.

(*) We wish to express our thanks to Merck and Co. for the supply of the necessary amounts of vitamin B_{12}.

EXPERIMENTAL

Experiments were performed in albino rats from our colony, feeding upon a diet deprived in factor N_1 for at least 90 days before starting the test. Rats were housed in individual cages with false bottom to prevent coprophagy. They had access to distilled water and to 10 % ethyl alcohol solution. The alcohol and the water intake was measured daily.

The deprived diet, given *ad libitum*, had the following composition:

Commercial casein	20 parts
Sucrose	70 „
Vegetable oil enriched in vitamins A, D and E (each ml containing 60 units of A, 10 units of D and 1 mg of alfa-tocopherol)	15 „
Saline mixture (Osborne and Mendel)	4 „

The following vitamin supplement dissolved in 2 ml of distilled water was given to each rat, every other day, by mouth:

Thiamin hidrochloride	0.04 mg
Riboflavin	0.05 „
Calcium pantothenate	0.05 „
Pyridoxine	0.02 „
Niacin	1.00 „
Choline chloride	2.00 „
Inositol	0.01 „
PABA	0.25 „
Folic acid	0.02 „
Biotin	0.01 „

The experiments in which vitamin B_{12} was injected were performed as follows: during 7 or 8 days, rats received subcutaneous injection of 0.9 ‰ sodium chloride solution; the following 7 or 8 days they received the injection of vitamin B_{12}, and during the next 7 or 8 days they were given a yeast supplement (*Torulopsis utilis*, dry 2g/100g rat/day) by mouth. When vitamin B_{12} was given by mouth, it was included in the vitamin supplement in doses of 10 μg every other day. This vitamin B_{12} supplement was maintained for 20 days. The alcohol intake will be expressed separating that of the first ten days from that of the ten following ones, to study if a transitory drop appears, as it has been observed by Brady and Westerfeld after giving different vitamin supplements (1). At the end of this 20 day-period, rats continued with the B_{12} supplement and received, in addition, dry liver in doses of 2g/100g rat/day by mouth, for ten days.

RESULTS AND DISCUSSION

Results are represented in figs. 1, 2 and 3. The results exhibited in figs. 1 and 2 show that in the cases in which B_{12} supplement was given subcutaneously in small doses, none of the rats decreased significantly the alcohol intake during the supplementary period. In one rat the intake of this period significantly differs from that of the control period, but the difference is not significant if compared with the intake during the immediately preceding period in which the rat received the injection of a saline solution.

Results summarized in fig. 3 show that in the cases in which vitamin B_{12} was given by mouth in higher doses, only in two out of eighteen rats the alcohol intake significantly decreased during the first ten days, but in both this low level was not maintained during the following ten days.

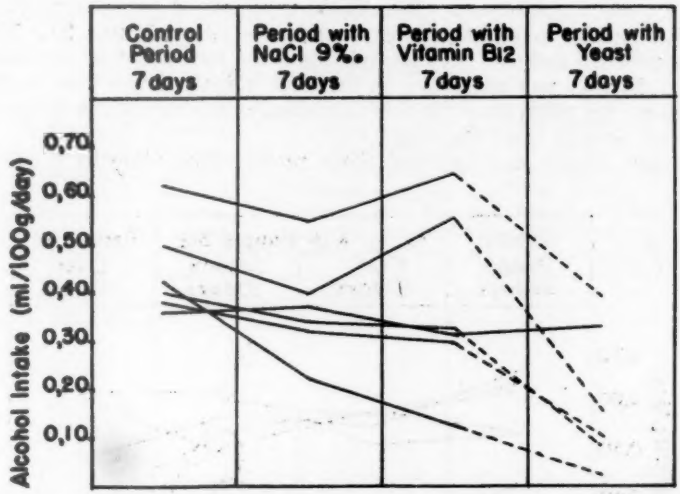

FIG. 1. — *Voluntary alcohol intake of N_1 deprived rats. During the second period the rats received subcutaneously* 0.1 *ml of a solution of* 0.9 *% NaCl daily. During the third period the rats received daily injections of* 0.1 *ml of the same solution containing* 0.23 *µg of vitamin* B_{12} *(Cobione Merck). During the last period the diet was supplemented daily with* 2*g of dried yeast (Torulopsis utilis) for eacn* 100 *g of body weight. Interrupted line joins points in which the difference is statistically significant at* 2 *% level.*

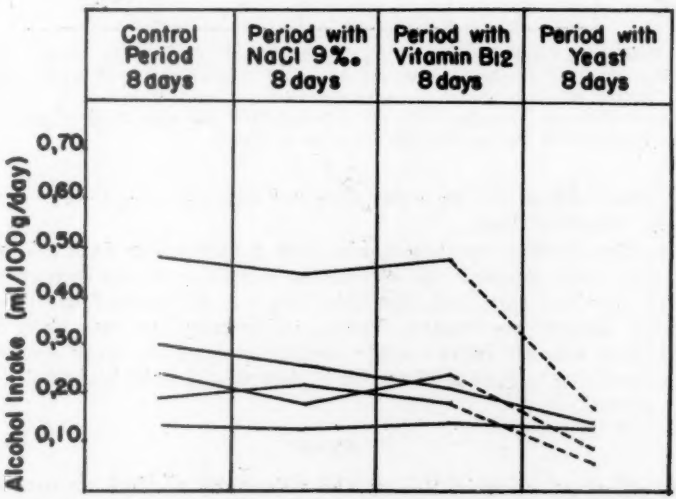

FIG. 2. — *Voluntary alcohol intake of N_1 deprived rats. Same explanation as in fig.* 1, *except for the amount of vitamin* B_{12} *supplement, that was in these cases* 0.46 *µg.*

The yeast supplement (*T. utilis*) produces a significant lowering of alcohol intake in eight out of eleven rats (figs. 1 and 2). The dry liver supplement reduced the alcohol intake in eleven out of eighteen rats (fig. 3). It was not necessary to test the effect of the liver and yeast supplements for more than ten days because, as we have already shown ([8]) this effect lasts at least 60 days.

We can clearly deduce from these results that vitamin B_{12} in doses

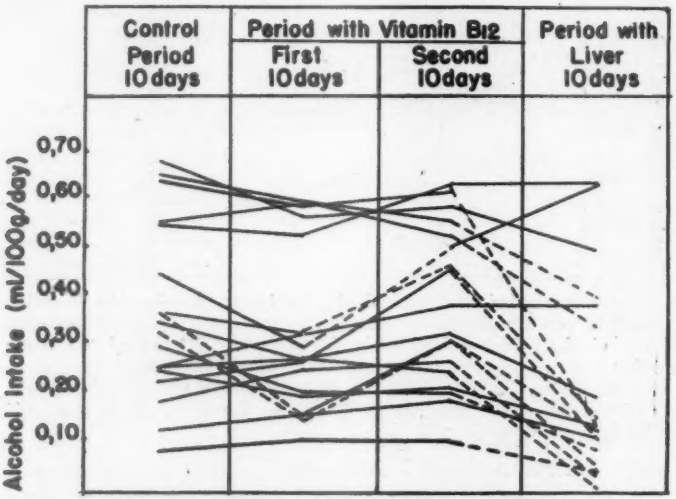

FIG. 3. — *Voluntary alcohol intake of N_1 deprived rats. During the period with vitamin B_{12}, the rats received by mouth 10 μg of this vitamin (Merck) every other day. During the last period the rats received the same supplement and in addition their diet was enriched with 2g of dried liver for each 100g of body weight, daily. Interrupted line means the same as in fig. 1.*

ranging from 0.23 μg to 5 μg a day does not diminish the alcohol consumption of N_1 deprived rats.

These results also confirm again that a factor (or factors), present in yeast and liver, descend the alcohol consumption in an important proportion of deprived rats, and that this factor is different from the eleven B vitamins already mentioned. The question whether rats that did not decrease their alcohol intake after receiving yeast or liver supplements were not sensitive to that addition or if they would need higher doses, still remains open.

SUMMARY

The effect of vitamin B_{12} on the voluntary alcohol consumption of rats maintained with a diet containing ten pure vitamins of the B complex were studied. No significant effect has been observed. Since supplementing the diet with liver or yeast produced a significant lowering

of alcohol intake in most of the same rats, it is concluded that factor N_1 and vitamin B_{12} are different substances.

REFERENCES

(1) BRADY, R., WESTERFELD, W. W.: *Quart. J. Stud. Alcohol.*, 1947, *7*, 499.
(2) MARDONES, J., HEDERRA, A., SEGOVIA, N.: *Bol. Soc. Biol. Santiago*, 1949, *7*, 1.
(3) MARDONES, J., ONFRAY, E.: *Rev. Chil. Hig. Med. Prev.*, 1942, *4*, 293.
(4) MARDONES, J., ONFRAY, E.: *Rev. Chil. Hig. Med. Prev.*, 1942, *5*, 17.
(5) MARDONES, J., SEGOVIA, N., HEDERRA, A.: *Bol. Soc. Biol. Santiago*, 1947, *4*, 121.
(6) MARDONES, J., SEGOVIA, N., HEDERRA, A.: *Bol. Soc. Biol. Santiago*, 1948, *5*, 27.
(7) MARDONES, J., SEGOVIA, N., HEDERRA, A.: *Bol. Soc. Biol. Santiago*, 1950, *7*, 59.
(8) MARDONES, J., SEGOVIA, N., HEDERRA, A.: *Bol. Soc. Biol. Santiago*, 1950, *7*, 82.
(9) MARDONES, J., SEGOVIA, N., ONFRAY, E.: *Arch. Biochem.*, 1946, *9*, 401.
(10) WILLIAMS, R. J., BERRY, L. J., BEERSTECHER, E. (JR.): *Proc. Nat. Acad. Sciences*, 1949, *35*, 265.

CONTRIBUTION TO THE STUDY OF VAGINAL SMEARS AND HORMONAL EXCRETION DURING NORMAL MENSTRUAL CYCLE

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SOME experimental studies have been reported on the levels of gonadotrophic and oestrogenic hormones in the blood and urine of normal women. Notwithstanding, it is difficult to know when hormonal excretion is normal in a given period of the cycle, since methods of hormonal extraction as well as biological assays and the kind of units employed vary according to the authors.

In 1933, Papanicolaou, was able to establish the pattern of the normal menstrual cycle by means of vaginal smears. Since then vaginal smears have been used for the study of ovarian disfunction, the action of gonadotrophic and sexual hormones on the genital tract and later, for cancer diagnosis ([27, 28, 37, 30]).

For clinical purposes several studies have been worked out. Endometrial biopsy ([6, 19, 39, 40]); endometrial biopsies and vaginal smears ([26]); vaginal smears ([27, 34]); hormonal determinations ([12, 7, 42, 19, 24, 31]); basal temperature and vaginal pH ([41, 4, 11, 5, 33]) etc.

The introduction of vaginal smears in routine examination has been suggested by several authors ([35, 2, 15, 16, 38]).

The scope of the present study is an attempt to correlate vaginal smears, which give an idea of the stage of the endometrium and ovarian function, with the pattern of the urinary gonadotrophin and oestrogens in different periods of the normal menstrual cycle.

MATERIAL AND METHODS

Vaginal smears.

The vaginal smears were taken daily by the patient, from the beginning of the flow until the first day of the next bleeding. In almost all cases two or more complete cycles were studied for each case. A month before and during the period the material was collected all hormonal treatment and douches were avoided.

The fluid was aspirated from the deepest part of the vagina with a glass pipette (15 cm in length by 0.5 cm in diameter) slightly curved and having a round opened tip in one of the extremities.

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* Head of Department: Dr .G. G. Villela.

The slides were prepared according to Papanicolaou's instructions (30). A typewritten sheet containing instructions for the withdrawal of material and preparation of slides as well as for the personal care was given to the patients. Sterilized pipettes, slides and bottles were supplied with.

For the study of vaginal smears, three slides were prepared and stained daily. The methods used for bacteriological, haematological and cytologic studies respectively were those of *Gram*, *Giemsa*, and Papanicolaou (29).

Vaginal pH.

In eighteen cases the vaginal pH was determined with the Universal pH indicator as proposed by Beilly (2).

The vaginal secretion was poured off from the glass pipette into a test tube with NaCl 0.85 %. Cases in which mucus was scanty a few ml of sterilized saline was injected through the vagina and aspirated to the test tube.

Basal temperature.

Soon after the vaginal smears collection, oral temperature was registered.

Length of the normal menstrual cycle.

Informative data on the length of 267 menstrual cycles and duration of bleeding, were recorded a month before and while hormonal determinations and vaginal smears were taken.

Urine collection.

The total 24 hours volume of urine was collected from the first day of bleeding until the onset of the next flow. According to previous experiments (17, 12, 8, 24, 32) the peak periods of gonadotrophic elimination vary from the 8th to the 12th and from the 18th to the 23rd day of the cycle. The urine voided in those days was kept in the ice box using acetic acid as a preservative for gonadotrophic determinations.

The urinary gonadotrophins were extracted by alcohol and acetone according to Bowman (23) or adsorbed on kaolin (24). Their activity was determined in immature female mice.

Gonadotrophic hormones. Preparation and assays.

Since it has been shown previously (24), that the peak of gonadotrophic hormone (stimulation fraction) occurred about the 8th to 12th day it was decided to carry out determinations on an aliquot of the pooled urine. The *stimulating hormone* was extracted according to the modified kaolin technique (24). Biological assays were performed with infantile (21-23 days old) female mice, 3 animals for each dose. A unit being considered the minimal dose injected which provoked a 100 % increase of the uterine weight with opening of the ostium.

The *luteinizing hormone*, was prepared from the pooled urine collected from the 18th to 23rd days of the cycle, according to Bowman's method, slightly modified (23). The biologcal assay was carried out on infantile female mice (23-25 days old) and the presence in the ovaries of two or more *corpora lutea* was considered a unit. Details of the techniques employed were given elsewhere (25).

OEstrogens.

The oestrogens were assayed in pooled specimens of urine collected in the intermenstrual period (13th-16th day) and from the 24th day to the end of the period. The urine was preserved with chloroform and placed in the ice box. Sometimes the length of time for urine collection, hormonal precipitation and biological assays, varied from 1 to 21 days.

Leiboff and Tamis technique (22), was used for the extraction the total oestrogenic hormones and the assay performed in spayed mice as in the classic Allen Doisy test, three animals for each dose. The results were given in mice units (M. U.).

In a few cases sodium pregnanediol glucuronidate excretion was estimated. The determinations were carried out in an aliquot of pooled urine collected from the 21st day of the cycle until the day preceding the menses. Venning's method modified by Astwood and Jones was used (1).

TABLE I

Phases of the cycle

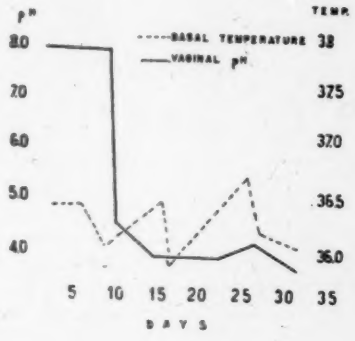
Cell types	Bleeding phase 1-7 days	Follicle stimulating phase 8-12 days	Oestrogenic phase 13-17 days	Luteinizing stimulating phase 18-23 days	Progestational phase 23rd day until bleeding
Blood red cells	Abundant	Sometimes appear to the end of period	Scanty		Appearing in the last two days
Polimorpho nuclears — Basophilic	Congregated into small clusters	Decreasing at the end of period	Rare or absent	Moderate	Usually present
Polimorpho nuclears — Acidophilic		The quantity varies greatly	Diminished or absent	Increased	Increasing at the end of period
Mononuclears	Large number			Scanty	In varying numbers
Histiocytes	Many around the end of period	Rare			
Superficial squamous type	Basophilic cells large and nucleated predominate as large sheets. As well as some small acidophilic cells with pyknotic nuclei	Acidophilic with light green cytoplasm and large nuclei	Elongated or navicular forms with pyknotic nuclei sharper outlines (Greenish yellow and pink)	Basophiles with large nuclei (brown). Acidophiles with granulated cytoplasm (green) and large nuclei (reddish)	Basophilic folded irregular cells with small nuclei (brown); acidophilic are scanty
Intermediate type	Rare	The number varies greatly	Large nuclei sometimes elongated	Some	Basophilic prevailing with elongated and central nuclei
Cornified cells	Absent		Pyknotic nuclei and basophilic cells with acidophilic granules scattered in the cytoplasm	Absent	Basophilic increased, acidophilic scanty
Endometrial cells	Basophilic small, round cells with dense and sometimes pyknotic nuclei with one or two nucleoli				Basophilic small round and nucleated
Mucus	Present with fibrinated appearance	Secretion during the outset of full development of cells	Present in early stage	Scanty or moderate	Increased with progressive fibrination

RESULTS

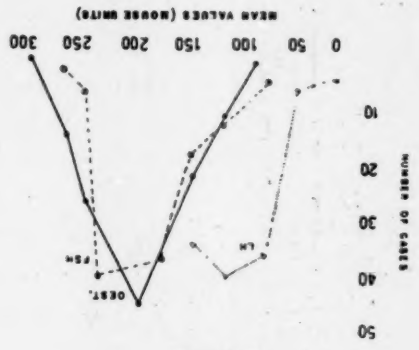
In the present paper, cycles are considered normal when the menstrual flow occurs without pain or psychosomatic disorders and at regular intervals.

Data in the length of cycle and on the duration of bleeding were available in 267 cycles. The length of the normal studied cycles ranged from 25-32 days, the greater frequency being observed between 26-29 days. Bleeding duration was 3-7 days; however the higher incidence was 3-5 days in duration.

No cycles longer than 32 days or shorter than 25 days were included in these studies. The highest percentage occurred between 27-30 days. These data indicate that the length of the cycle and time of bleeding although correlated with other physiological functions besides the hormonal ones, are fairly constant.



Graph 1.



Graph 2.

GRAPH 1. — *Vaginal pH and basal temperature during the menstrual cycle.*

GRAPH 2. — *Hormonal elimination in normal menstrual cycles during the intermenstrual (oest.), follicle stimulation (FSH) and luteinizing stimulating (LH) phases. Mean values obtained from* 128 *normal cycles.*

Cytology.

Normally vaginal smears contain cells of the superficial type and red blood cells, histiocytes and leucocytes as well as bacterial bodies and mucus. According to the phase of the cycle, acidophilic or basophilic cells appeared presenting different shapes with nucleal modifications. A detailed account of all modifications observed is unnecessary because the different patterns of cells which appeared in the various stages of the cycle are condensed in table I.

It is difficult to recognize the stage of the cycle by means of one single vaginal smear; however, when several normal cycles have previously been studied, it is possible to determine the phase of the cycle with a few slides if the time of bleeding and length of the cycle are known.

The pH of the vaginal secretion ranged from 3.2 to 7.8 and the mean values are included in graph 1.

The relationship between vaginal smears and hormonal excretion has been investigated and the mean values for gonadotrophic and oestrogenic hormones are represented in graph 2.

Information concerning the *pregnanediol glucuronidate* excretion was obtained in only eleven cases. The total excretion of the cycle (21st day until the bleeding) was from 5-20 mg and the values ranged between 3-9 mg per liter of urine. There is a close relationship between the cytological pattern and hormonal excretions in almost all cycles.

Basal temperature was recorded in eleven cycles, and their correlation to the time of ovulation observed by means of the vaginal smears. In 8 cases the temperature rose between the 11th and 14th days of the cycle and in 3 of them some pain occurred. Temperature fluctuations were closely related with the pain and the blood detection in the smears (graph 1).

Soon after the appearance of blood, the temperature decreased significantly.

Discussion

The purpose of these studies was to evaluate the best way of determining the ovarian function in a given period of the menstrual cycle by means of a single assay.

Comparative cytological and hormonal determinations were carried out on 128 cycles; their length was determined on 267 cycles. The mean value found for the length of normal cycles was 28.4 days. Cycles longer than 28 days showed oestrogenic bleeding and the oestrogenic stage was later (14-16th days). It seems that there is some correlation between the length of the cycle and the hormonal excretion.

In a few number of cases we have correlated vaginal pH and basal temperature with blood detection in the vaginal smears. Although the number of observations is limited it seems to us that these data considered at the same time permit one to determine the time of ovulation.

Since 1904 it has been known that the body temperature is correlated with gonadal function and varies during the menstrual cycle (Van den Velde [41]). However, only many years later the exact relationship between gonadal function and body temperature was established and became a useful method of determining the time of ovulation ([20, 34, 9]). Rubenstein ([35]) observed the low point in the basal rectal temperature curve, when the follicles mature, i. e., just before ovulation. He assumes that the temperature curve is particularly useful when correlated with vaginal smears.

The temperature curve as a mean of ascertaining the time of ovulation has been tried by several authors (D'Amour [9]; Goldzieher [11]; Diddle [10], etc.). In 1949, Krohn called attention to the close relationship between the temperature changes and the *"Mittelschmertz"* occurrence. As the evidence suggests the pain is connected with the ovarian changes

either by follicle distensión just before ovulation or by the rupture of the follicle ([21]). Our results confirm Krohn's statement (graph 1).

It is posible to establish a relationship between vaginal smears and hormonal levels during the menstrual cycle. Rubenstein and Duncan ([36]), carried out studies on five patients, and concluded that the evaluation of vaginal smears permits only an approximate idea of the degree of vaginal proliferation and that the urinary oestrogens assay is also an approximative technique. This means that the human vaginal smears may be used as an approximative method for the oestrogens production but it is not proportional to the oestrogens excretions.

Haam ([14]), has studied biopsies, vaginal smears, gonadotrophic and oestrogenic determinations during the menstrual cycle. He stated that the vaginal smears are of a great value only in amenorrheic cases while it is of no value in normal menstruating women. D'Amour ([9]) evaluated the validity of the test for determining the time of ovulation and he agrees that there is a close relationship between the results of hormonal assay (gonadotrophic peak in the mid-interval), vaginal smears and time of ovulation.

Some discrepancies have been observed in our own observations; from the studied cycles, 18 showed no correlation between hormonal excretion and vaginal patterns. The hormonal elimination was very low in urine and blood, though vaginal smears were fairly normal. In 3 cases gonadotrophic hormones in urine presented high levels and the smears were normal. In one case, studied during 3 cycles, the oestrogenic hormones were absent in urine, vaginal smears were fairly normal (cornified cells appeared only on 17-18th day of the cycle of 32 days) but oestrogens were increased in the blood (150 M.U.).

These results show that other factors besides endometrium are involved. It is difficult to establish with a single hormonal determination or by a single slide that a given cycle is normal, because some other hormonal metabolism factors must be considered.

The numerous reports on the subject are the best evidence of its difficulty.

Conclusions

The analysis of our results permits the following conclusions:

a) When the time of bleeding is known it is possible to determine the stage of the cycle by means of vaginal smears if they are taken in 3 - 5 consecutive days. They are a useful help for the therapeutic control.

b) Gonadotrophic hormones concentration varied largely. The lowest value recorded for the stimulating fraction (8-12th day) was 80 M.U. and the highest 270 M.U. per liter; the luteinizing fraction determined from 18-23 days, ranged from 20-150 M.U. per liter.

c) Total estrogens were evaluated in the midinterval time and the values recorded were 90-300 M.U. (Allen Doisy) per liter. All these determinations were carried out on an aliquot from the pooled urine.

Hormonal determinations are very important in the diagnosis of ovarian function when aided by vaginal smears, because hormonal excre-

tions alone may be influenced by other physiological factors which interfere with their metabolism, whereas vaginal smears indicate the degree of hormonal utilization. When both techniques are employed at the same time, it is possible to evidenciate the metabolic disorders.

d) Gonadotrophic stimulating hormone was higher and total oestrogens excretion lower, when cycles were of 25 days.

In cycles of 30-32 days a lengthening of the oestrogenic action was observed in vaginal smears varying from 3 to 5 days (13-18th day of the cycle). The peak excretion was observed between the 16th-18th days (3 studied cycles).

These data are closely related to ovarian function. Hormonal determinations and cytologic study enable us to know the degree of ovarian function when a careful study of them has been made during a large number of cycles. It is possible to recognize the stage of the cycle during a given period by means of a few slides and a single hormonal determination if other data are established previously.

REFERENCES

(1) ASTWOOD, E. B., JONES, G. E. S.: *J. Biol. Chem.*, 1941, *137*, 397.
(2) BEILLY, J. S.: *Endocrinol.*, 1940, *26*, 959.
(3) BOWMAN, D.: *J. Biol. Chem.*, 1941, *137*, 293.
(4) BUXTON, CH. L., ATKINSON, W. B.: *J. clin. Endocrinol.*, 1948, *8*, 544.
(5) BUXTON, CH. L., ENGLE, E. T.: *Amer. J. Obst. Gynec.*, 1950, *60*, 539.
(6) CAMPBELL, R. E.: *Surg. Gynec. Obst.*, 1936, *63*, 734.
(7) D'AMOUR, F. E., FUNK, D., LIVERMAN, H.: *Amer. J. Obst. Gynec.*, 1939, *37*, 940.
(8) D'AMOUR, F. E., WOODS, L.: *J. Clin. Endocrinol.*, 1941, *1*, 433.
(9) D'AMOUR, F. E.: *J. Clin. Endocrinol.*, 1943, *3*, 41.
(10) DIDDLE, A. W.: *Amer. J. Obst. Gynec.*, 1948, *56*, 537.
(11) GOLDZIEHER, J., HENKIN, A., HAMBLEN, E.: *Amer. J. Obst. Gynec.*, 1947 *57*, 668.
(12) GUSTAVSON, R., MASON, L., HAYS, E., WOOD, T., D'AMOUR, F.: *Amer. J. Obst. Gynec.*, 1938, *35*, 115.
(13) GREENBLATT, F.: *J. M. A. Georgia*, 1941, *30*, 297.
(14) HAAM (VON), E., ROTHERMICH, N. O.: *Proc. Soc. exp. Biol.*, N. Y., 1940, *44*, 369.
(15) HAMBLEN, E., ANGLER, W., BAPTIST, M.: *Amer. J. Obst. Gynec.*, 1942, *44*, 442.
(16) HAMBLEN, E., HIRST, D., ANGLER, W., DURHAM, N.: *Amer. J. Obst. Gynec.*, 1943, *45*, 268.
(17) HELLER, C., HELLER, E.: *J. Clin. Invest.*, 1939, *18*, 171.
(18) HELLER, E.: *J. Clin. Endocrinol.*, 1941, *1*, 813.
(19) ISRAEL, S., MAZER, C.: *Amer. J. Obst. Gynec.*, 1938, *36*, 445.
(20) KURZROK, R., KIRMAN, I., CREELMAN, M.: *Amer. J. Obst. Gynec.*, 1934, *28*, 319.
(21) KROHN, P.: *Brit. Med. J.*, 1949, n 4609, 803.
(22) LEIBOFF, S., TAMIS, A.: *J. lab. clin. Med.*, 1938, *24*, 178.
(23) MELLO, M. I.: *Rev. Brasil. Biol.*, 1943, *3*, 119.
(24) MELLO, M. I.: *Mem. Inst. Osw. Cruz*, 1943, *39*, 419.
(25) MELLO, M. I.: *Rev. Ginec. d'Obst.*, 1950, *44*, 1.
(26) NEUSTADTER, T., MACKENZIE, L.: *Amer. J. Obst. Gynec.*, 1944, *47*, 81.
(27) PAPANICOLAOU, G.: *Amer. J. Anat.*, 1933, *52*, 519.
(28) PAPANICOLAOU, G., SHORR, E.: *Amer. J. Obst. Gynec.*, 1936, *31*, 806.
(29) PAPANICOLAOU, G.: *Science*, 1942, *95*, 438.
(30) PAPANICOLAOU, G., TRAUT, H. F.: *Diagnosis of uterine cancer by the vaginal smear.* Humphrey Milford, Oxford University Press, 1943.
(31) PEDERSEN-BJERGAARD, K., TONNESEN, M.: *Acta Endocrinol.*, 1948, *1*, 38.
(32) PEDERSEN-BJERGAARD, G., PEDERSEN-BJERGAARD, K.: *Acta Endocrinol.*, 1948, *1*, 263.
(33) POMMERENKE, W., VIERGIVER, E.: *Amer. J. Obst. Gynecol.*, 1947, *54*, 676.

(34) RUBENSTEIN, B.: *Amer. J. Physiol.*, 1937, *119*, 635.
(35) RUBENSTEIN, B.: *Endocrinol.*, 1940, *27*, 843.
(36) RUBENSTEIN, B., DUNCAN, D.: *Endocrinol.*, 1941, *28*, 911.
(37) SHORR, E., PAPANICOLAOU, G.: *Proc. Soc. exp. Biol.*, N. Y., 1939, *14*, 629.
(38) SIEGLER, S., BAUER, D.: *Amer. J. Obst. Gynec.*, 1943, *45*, 277.
(39) STURGIS, S.: *A. J. Surg.*, 1936, *33*, 369.
(40) TRAUT, H.: *Surg. Gynec. Obst.*, 1935, *61*, 145.
(41) VELDE, VAN DEN, T.: *Ueber den Zusammenhang zwischen Ovarialfunktion, Wellenbewegung und Menstrualblutung.* Haarlen, 1904.
(42) WERNER, S.: *J. clin. Invest.*, 1941, *20*, 21.

GRAFTING OF KIDNEYS FROM RATS WITH DESOXICORTICOSTERONE HYPERTENSION INTO NORMOTENSIVE RECEPTORS *

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IN RECENT years the mechanism by which desoxycorticosterone acetate (DCA) induces hypertension has been the subject of extensive work (cf. Braun-Menéndez and Prado, 1950; Friedman, Friedman and Nakashima, 1951; Hall and Hall, 1951; Masson, Corcoran and Page, 1951; Prado, 1950; Selye, 1951; Silva and Croxatto, 1950). The possibility that this hypertension might be of renal origin has been suggested although no definite evidence has been given to support this contention. Houssay and Fasciolo (1937), using the technique of grafting, by vascular anastomosis, an ischemic kidney into the cervical region of a receptor dog, were able to demonstrate conclusively that a vasoconstrictor substance is liberated from the ischemic kidney. We decided to apply such experimental technique to the rat using as a hypothetical source of renin or of vasoconstrictor substances, the kidney of rats in which hypertension produced by implantation of pellets of desoxycorticosterone acetate had been previously developed. Normotensive rats were employed as receptors.

METHODS

Production of hypertension. — DCA-hypertension was obtained by implanting two pellets of 20 mg of DCA (1) into male, castrate, albino rats, 60-80 g of body weight, nephrectomized at right, and keeping the animals on one per cent sodium chloride solution as drinking fluid. Under these conditions, it has been our experience (Prado, 1950; Herbert-Carrington, Magaldi and Prado, 1950) that DCA-hypertension is well developed twenty days following the implantation. To follow the modifications of blood pressure during the development of DCA-hypertension the pletysmographic method of Williams, Harrison and Grollman (1939) was employed, by using the very convenient apparatus described by Magaldi (1945).

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(1) The DCA used was kindly supplied by Productos Químicos Ciba, S. A., Laboratórios Silva Araujo-Roussell, S. A., and Schering Produtos Químicos e Farmacêuticos S. A.

Preparation of the kydney donor. — Under pentobarbital (50 mg/kg intraperit.) anesthesia the vessels around the renal pedicle, including some two centimeters of the aorta and cava vein below the renal artery and vein, are well exposed. A very rapid and clean exposure of the whole region is obtained, by pressing the middle line with two large cotton pads, between thumb and forefinger, and then pulling them to the sides; hemorrhages which interfere with the success of the operation are thus avoided. Further cleaning and isolation of the vessels is done with pieces of cotton held with small forceps. A thread is now passed under the cava and aorta, *immediately above* the renal artery and vein. All the branches from the aorta and

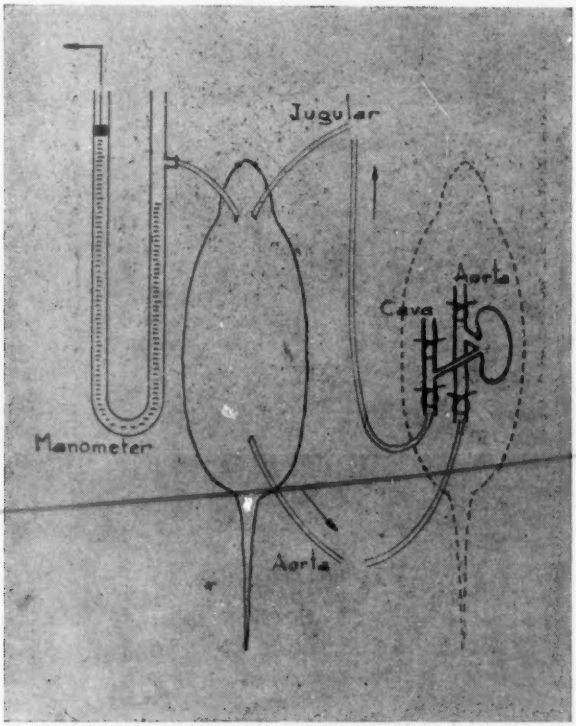

FIG. 1. — *Schematic diagram showing the vascular ligatures and anastomosis necessary to connect the kidney into the general circulation of a receptor rat.*

cava below the renal pedicle down to a distance of two centimeters are ligated, special care being given to two or three small branches from the cava which come from the dorsal muscles and which may not be seen if not especially looked for. The adrenal pedicle is also ligated. After all the vascular ligatures are ready, the aorta and cava are ligated approximately two centimeters caudad to the renal pedicle. A *second thread* is now passed under these vessels, *below* the renal pedicle. Interrupting the circulation momentaneously in the aorta by pulling the *first thread*, a small incision is made on the vessel and a plastic tube (3) of appropriate diameter, full of

(3) Polyethylene plastic tubes (Clay, Adams, Co. U.S.A.).

heparin solution and plugged with a pin is inserted and firmly tied in the aorta using the *second thread.* To cannulate the cava, the circulation is interrupted a few minutes also by pressing with a piece of cotton the confluence of the renal vein with the cava. It is always more difficult to insert the plastic tube in the vein because it collapses completely as soon as it empties as a result of the incision necessary to cannulate it. It is advisable to make a small incision, just sufficient to insert the tip of the plastic cannula and then force its passage through the small incision.

During the interruption of circulation in the aorta and cava there occur short periods of renal ischemia which, however, do not produce a rise in the blood pressure of the receptor as will be shown later.

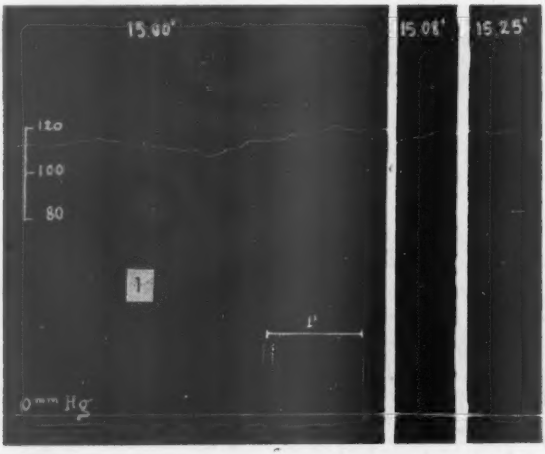

FIG. 2. — *Rat's carotid blood pressure after pentobarbital (10 mg intraperitoneally) and ether. At 1 grafting, by vascular anastomosis, of a normal kidney. No conspicuous changes of the blood pressure.*

Preparation of the receptor rat. — As receptors, normal, adult rats, 200-300 g of body weight, were used. The receptor is bilaterally nephrectomized six hours previously in order to increase its sensitivity to renin. At the time of the grafting procedure, the receptor under pentobarbital anesthesia, is attached on its back on the operating board. The right jugular vein is exposed without insisting on a thorough isolation (which collapses the vein completely) and threads are passed around it with a needle. Following ligation of the cranial side, a plastic tube, full of heparin solution and plugged with a pin, is inserted deeply into it through a small incision and tied. The carotid artery is isolated and cannulated with a plastic tube which will be subsequently connected with a mercury manometer for registration of the blood pressure. Thirdly the aorta of the receptor is carefully isolated and also cannulated. Now the animal is heparinized through the jugular vein and is ready to be employed as the recipient rat.

Kidney grafting. — Using plastic tubes of slightly different internal diameters it is easy to make connections between the kidney preparation (from the donor) and the receptor by simply inserting the smaller into the larger tubes. By pulling a thread left around the arteries it is easy to make the plastic connections without hemorrhages. Following a previous control period of blood pressure registration the kidney to be grafted is connected with the receptor as follows. The threads left on the aorta and cava of the donor are ligated above the renal artery and vein. The plastic tube in the aorta of the receptor is *immediately* connected with the plastic in the aorta of the donor; and the plastic in the cava of the donor is joined with the

FIG. 3. — *Carotid blood pressure of a rat with renal ischemia of 6 h 40 m, duration. Pentobarbital and ether anesthesia.* 1) *Removal of the clamp followed by increase of the blood pressure.* 2) *Renal pedicle clamped again. Note slow drop of the blood pressure.*

FIG. 4. — *Carotid blood pressure of a rat with renal ischemia of 6 h 20 m, duration. Pentobarbital and ether anesthesia.* 1) *Removal of the clamp.* 2) *New clamping.* 3) *Second release of the clamp. Note tachyphylaxis.*

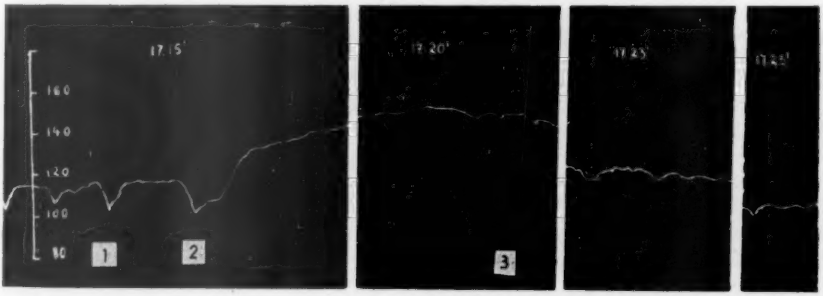

FIG. 5. — *Rat's carotid blood pressure. Pentobarbital and ether anestesia. Result of grafting a kidney in total ischemia during 6 h 30 m.* 1) *Vascular anastomosis by connection of the plastic tubes.* 2) *Clamp removed from donor's renal pedicle.* 3) *Renal pedicle clamped again.*

plastic in the right jugular vein of the receptor (fig. 1). When all the vascular anastomosis are ready the blood of the receptor animal circulates through the kidney of the donor and returns through the renal vein, cava and respective plastic to the right jugular vein of the receptor. If the return of blood is avoided, by plugging the plastic in the cava no drop of the blood pressure of the donor should occur if the kidney preparation *is not leaking*. Indication that the grafted kidney is well perfused is gained from: a) change of color of the perfused organ; b) by introducing a small air bubble at the connection of the plastic tubes or c) by injecting epinephrine in the perfused kidney which will raise the blood pressure of the receptor.

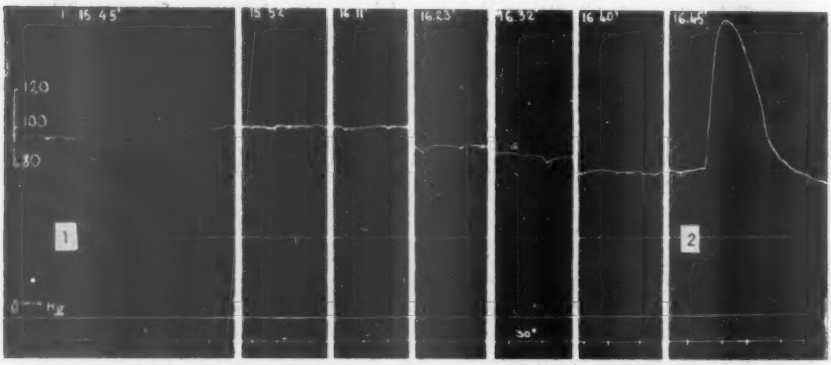

FIG. 6. — *Rat's carotid blood pressure. Pentobarbital and ether anesthesia. Result of grafting a DCA-hypertensive-kidney.* 1) *Connection of the plastic tubes. There was no elevation of the recipient's blood pressure even after 50 minutes of perfusion.* 2) *Injection of 2 μg of epinephrine into the grafted kidney.*

RESULTS

a) *Grafting of normal kidneys.* — Kidneys grafts from six normal rats were not followed by modification of the recipient's blood presure (fig. 2).

b) *Ischemic kidneys.* — Reestablishement of circulation through a kidney maintained in total ischemia has been proved to produce elevations of the blood pressure in the rat (Lewis, Leo and Prinzmetal, 1941). We have been able to reproduce these observations; under our experimental conditions, clamping of the kidney pedicle for a period of 4 to 6 hours was always followed, upon reestablishment of the circulation, by an unmistakable rise of the blood pressure. In six experiments the blood pressure rose 30 to 75 millimeters of mercury within aproximately five minutes (table I). If the renal pedicle is clamped again the blood pressure drops slowly (fig. 3) and a second release of the clamp is followed by a much smaller or no rise at all of the blood pressure (fig. 4). Renal venous blood collected from such kidneys has a strong vaso-constricting

TABLE I

Effect of removing the clamp from the renal pedicle of a totally ischemic kidney on the blood pressure of the rat.

Duration of kidney clamping	Blood pressure before releasing clamp *mm/Hg*	Maximum blood pressure following release clamp *mm/Hg*	Maximum difference in blood pressure *mm/Hg*
0 h 25 m	90	90	0
0 h 30 m	80	86	+ 6
0 h 48 m	52	56	+ 4
1 h 18 m	50	54	+ 4
5 h 20 m	130	160	+ 30
5 h 30 m	70	110	+ 40
6 h 00 m	90	150	+ 60
6 h 20 m	110	150	+ 40
6 h 30 m	90	140	+ 50
6 h 40 m	80	155	+ 75

TABLE II

Effect of grafting a previously ischemic kidney on the blood pressure of a recipient nephrectomized rat.

Duration of ischemia	Blood pressure before grafting *mm/Hg*	Maximum blood pressure following grafting *mm/Hg*	Maximum difference in blood pressure *mm/Hg*
4 h 0 m	120	130	+ 10
6 h 0 m	90	128	+ 38
6 h 0 m	60	100	+ 40
6 h 30 m	110	150	+ 40
7 h 0 m	70	120	+ 50
7 h 30 m	92	118	+ 26
8 h 0 m	80	120	+ 40

TABLE III

Effect of grafting a DCA-hypertensive-kidney on the blood pressure of a recipient nephrectomized rat.

Approximate duration of DCA-hypertension *days*	Blood pressure following graft *mm/Hg*	Time of circulation through graft *min.*	Maximum blood pressure following graft *mm/Hg*
15	110	20′	110
15	64	15	62
30	100	15	102
30	90	30	90
30	100	43	110
70	94	50′	100

action on the toad Läwen-Trendelenburg preparation (Prado, Paiva, Valle and Prado, 1951). Clamping of the renal pedicle for short periods, up to 78 minutes, was not followed by a significant blood pressure rise when circulation was reestablished (table I).

c) *Grafting of ischemic kidneys.* — Grafting of seven kidneys which had been in complete ischemia for periods of 4 to 8 hours was followed, in 6 instances, by rises of the recipient's blood pressure from 26 to 50 millimeters of mercury. In one experiment only a small rise was noticed. Table II shows the results of all the experiments and fig. 5 represents one of them.

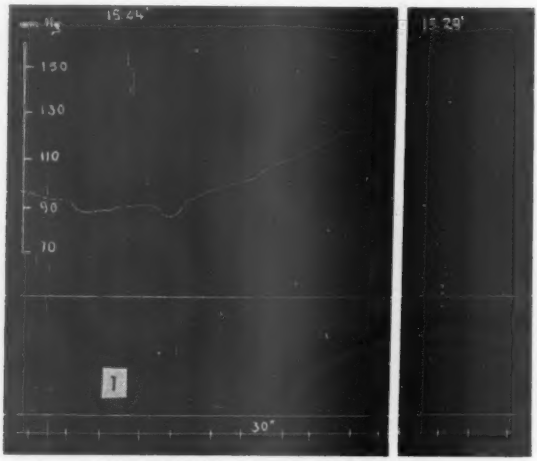

FIG. 7. — *Carotid blood pressure of a DCA-hypertensive-rat after pentobarbital and ether anesthesia.* 1) *Removal of the clamp kept on the renal pedicle for 5 hours. The normotensive level of the blood pressure previous to the clamp removal is probably due to hemorrhages occurred during the clamping period.*

d) *Grafting of DCA-hypertensive-kidneys.* — Rats with hypertension of a duration ranging from 15 to 70 days approximately were used. In six experiments, in which the grafted kidney was perfused during periods varying from 15 up to 50 minutes, *no significant elevation* of the recipient's blood pressure was observed. A typical result is recorded in figure 6 which shows the blood pressure of a recipient whose graft was actually perfused during 50 minutes. Other experiments may be seen in table III.

It has been observed, however, that the kidney of a DCA-hypertensive-rat may liberate large amounts of renin when submitted to total ischemia of a few hours duration (fig. 7). Hemorrhages always occurred during the period of 4 to 6 hours in which the renal pedicle remained

clamped, thus bringing the blood pressure to normotensive levels, therefore in the few experiments performed it was not possible to maintain the hypertensive level of blood pressure previous to the releasing of the clamp.

Discussion

The experiments here described show that grafting DCA-hypertensive-kidneys into normotensive receptors do not rise the recipient's blood pressure. That this technique is able to demonstrate the presence of large amounts of vasopressor substances in renal venous blood is demonstrated by the increases in blood pressure observed when totally ischemic kidneys are the grafted ones. One cannot conclude definitely, however, on the basis of the reported experiments, that the DCA-hypertensive-kidney does not release vasopressor substances. We have observed (Prado, Paiva, Valle and Prado, 1951) that blood collected from the renal vein of DCA-hypertensive-animals do contain renin. Even when special care is taken to avoid hypotension during the blood collection, as was the case in our experiments, the incubation of such a blood with hypertensinogen produces hypertensin, as evidenced by its vaso-constricting activity on the Läwen-Trandelenburg toad's preparation.

In agreement with Braun-Menéndez, Covian and Rapela (1947), however, we have observed that blood collected from the renal vein of normal rats also contain renin. Estimation of renin released by the DCA-hypertensive-kidney as compared with the normal, became therefore imperative. Unfortunately the method of Fasciolo and Taquini (1947), when applied to *Bufo ictericus* Spix (4) did not permit to answer this question because the preparation was unsuitable to quantitatively estimate hypertensin. Other methods of renin assay have to be tried.

On the other hand the role played by extrarenal factors in the genesis of DCA-hypertension (Braun-Menéndez and Prado, 1950; Raab, Humphreys and Lepeschkin, 1950), seem to merit now further investigation.

It is important to emphasize that whichever the mechanism through which DCA *initiates* hypertensive disease, the self- sustained hypertension following withdrawal of treatment (Friedman and Friedman, 1949; Herbert-Carrington, Magaldi and Prado, 1950; Friedman, Friedman and Nakashima, 1950) may have an entirely different explanation.

Summary

A technique has been described by which it has become possible to graft a rat's kidney into a recipient rat for periods up to one hour. Using such a technique it was shown:

1) The grafting of normal kidneys did not modify the recipient's blood pressure in six experiments;

(4) Cf. *M. P. Sawaya* (1949). Sôbre o nome do sapo comum no Estado de São Paulo. *Ciência e Cultura* 1: 38.

2) In six out of seven grafting of kidneys which had been in total ischemia for periods ranging from 4 to 8 hours, a marked elevation (26 to 50 mm Hg) of blood pressure was produced in the receptor;

3) Kidneys from rats with recent (15-70 days) hypertension due to implantation of desoxycorticosterone acetate pellets produced no significant rise of the recipient's blood pressure. This was observed in all the six experiments performed, transfusing the grafted kidneys during periods ranging from 15 up to 50 minutes.

4) If DCA-hypertensive-kidneys have its pedicle clamped during a few-hours, they will liberate renin upon reestablishment of the circulation.

BIBLIOGRAPHY

BRAUN-MENÉNDEZ, E., COVIÁN, M. R., RAPELA, C. E.: *Rev. Soc. Argent. Biol.*, 1947, *23*, 131.

BRAUN-MENÉNDEZ, E., PRADO, J. L.: *Rev. Soc. Argent. Biol.*, 1950, *26*, 188.

FASCIOLO, J. C., TAQUINI, A. C.: *Rev. Soc. Argent. Biol.*, 1947, *23*, 138.

FRIEDMAN, S. M., FRIEDMAN, C. L.: *Canad. M. A. J.*, 1949, *61*, 596.

FRIEDMAN, S. M., FRIEDMAN, C. L., NAKASHIMA, M.: *J. Exper. Med.*, 1951, *93*, 361.

HALL, C. E., HALL, O.:*Arch. Path.*, 1951, *51*, 249.

HERBERT-CARRINGTON, J. R., MAGALDI, J. B., PRADO, J. L.: *Ciência e Cultura*, 1950, *2*, 282.

HOUSSAY, B. A., FASCIOLO, J. C.: *Rev. Soc. Argent. Biol.*, 1937, *13*, 284.

LEWIS, H. A., LEO, S. D., PRINZMETAL, M.: *Amer. Heart. J.*, 1941, *21*, 319.

MAGALDI, J. B.: *Hipertensão arterial experimental do rato. Tese de doutoramento.* São Paulo, Emp. Gráfica "Revista dos Tribunais" Ltda., 1945.

MASSON, G. M. C., CORCORAN, A. C., PAGE, I. H.: *J. lab. clin. Med.*, 1951, *38*, 213.

PRADO, E. S., PAIVA, A. C. M., VALLE, J. R., PRADO, J. L.: (Unpublished results, 1951).

PRADO, J. L.:*Estudos sôbre hipertensão hormonal experimental. Thesis presented to Escola Paulista de Medicina.* São Paulo, Impressora Ipsis, 1950.

RAAB, W. R. J., LEPESCHKIN, H., LEPESCHKIN, E.: *J. clin. Invest.*, 1950, *29*, 1397.

SELYE, H.: *1st. Annual Report on Stress.* Montreal, Acta Inc. Medical Publishers,

KIDNEY AND ALLOXAN

Researches on their rôle in the production of alloxanic diabetes

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IN 1946 Jiménez Díaz, Grande and Oya [5] showed that the temporal circulatory exclusion of the kidney by clamping off its vascular pedicle during the injection of a dose of 80 to 100 mg of alloxan per kg of body weight prevented the development of the severe diabetes, which, together with the uremic syndrome, is the usual consequence of the injection of such doses of alloxan.

The authors assumed that such a protection could be due either to the necessity of contact of the alloxan with the kidney in order to develop its diabetogenic action, or to the existence of a renal injury caused by the alloxan which would condition its diabetogenic effects.

More recently, these same autors have adduced new arguments in favour of the participation of the kidney in the production of alloxanic diabetes [6, 7], concluding that the renal lesion aggravates alloxanic and pancreatic diabetes. They also insist on the participation of the kidney in carbohydrate metabolism.

The work of Duff and collaborators [2], of Gold [3], Martínez, Gitter and Covián [8] and of Houssay and Martínez [4], give different results and interpretations.

Except Houssay and Martínez, who worked on dogs, the remaining authors could not verify on rabbits and rats the protective effect described by Grande and his collaborators.

Houssay and Martínez corroborated that if the alloxan was injected during the clamping of the renal pedicle, a lesser number of diabetic animals was obtained; but the same results were obtained by clamping only the renal nerves or the vessels of the splenic pedicle. They conclude that the presence of the kidney is not necessary to produce the alloxanic diabetes, and that the protective effect of the clamping of the renal vessels is the result of a reflex that influences the pancreatic circulation, giving evidence of the existence of such a reflex.

In a very recently published paper [1] the Spanish authors debate the researches of Houssay and his collaborators. Though they confirm

TABLE I

Effect of alloxan on the glycemia of normal dogs anaesthetized with ether.

Dog	Alloxan mg/kg.	Blood sugar mg per cent. Time in days 0	1	2	3	4	5	6	7	8	9	10	12	Survival time (days)
1	80	95	97	253	255	280	326	270	255	198	334	300	340	—
2	80	94	168	446	474									3
3	80	92	58	161	125	145	156	225	250	267	450			9
4	80	102	215	175	210	240	420	410	480					7
5	80	90	155	130	136	200	370	420	440					7

TABLE II

Effect on glycemia of the injection of alloxan in the left renal artery in dogs with previous clamping of a renal pedicle and occlusion of the pedicle of the injected kidney fifteen seconds after having performed the injection.

Dog	Alloxan mg/kg.	Blood sugar mg per cent. Time in days 0	1	2	3	4	5	6	7	8	9	10	18
6	80	100	86	98	110	90	94	—	98	99	86	98	87
7	80	90	100	102	130	132	104	90	94	104	98	102	92
8	80	105	105	100	100	90	94	85	88	98	80	82	98
9	80	100	101	90	93	108	87	80	108	79	95	85	79
10	80	98	120	—	102	94	95	71	82	88	88	102	93
11	80	88	70	84	85	87	95	94	71	82	80	82	80

TABLE III

Effect of alloxan on the glycemia of dogs with clamping of the hepatic vessels.

Dog	Alloxan mg/kg.	Blood sugar mg per cent. Time in days 0	1	2	3	4	5	6	7	8	9	10	17
12	80	101	71	93	87	87	100	80	72	80	81	86	—
13	80	85	68	99	89	94	99	117	111	107	101	100	103
14	80	91	102	105	102	102	80	94	99	96	94	88	80
15	80	105	107	116	111	96	94	97	104	114	101	105	105
16	75	96	96	95	80	102	91	98	97	89	86	86	80
17	75	93	93	91	95	88	87	85	87	80	86	80	85

their results, they consider that there is not yet enough evidence to admit the pancreatic reflex and that even if alloxanic diabetes can be influenced by the reflex way this does not exclude the rôle of kidney in the origin of alloxanic diabetes.

We are giving now the results of our observations.

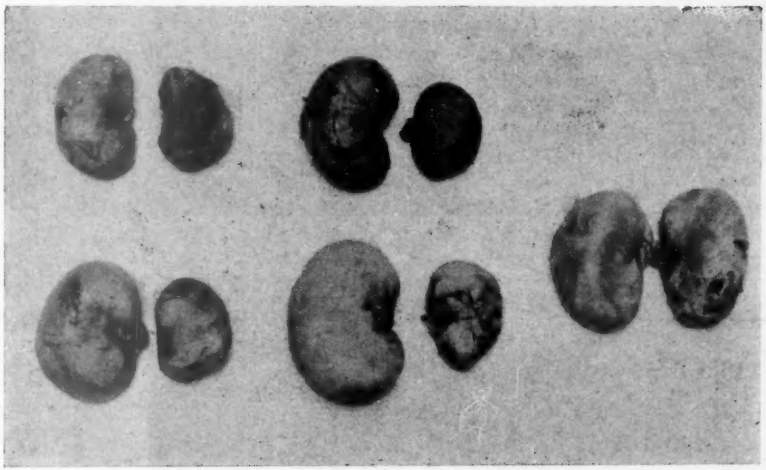


FIG. 1. — *Kidneys of dogs of group II, in which alloxan was injected in a branch of their left renal artery, while the pedicle of the right kidney was clamped.*

MATERIAL AND METHODS

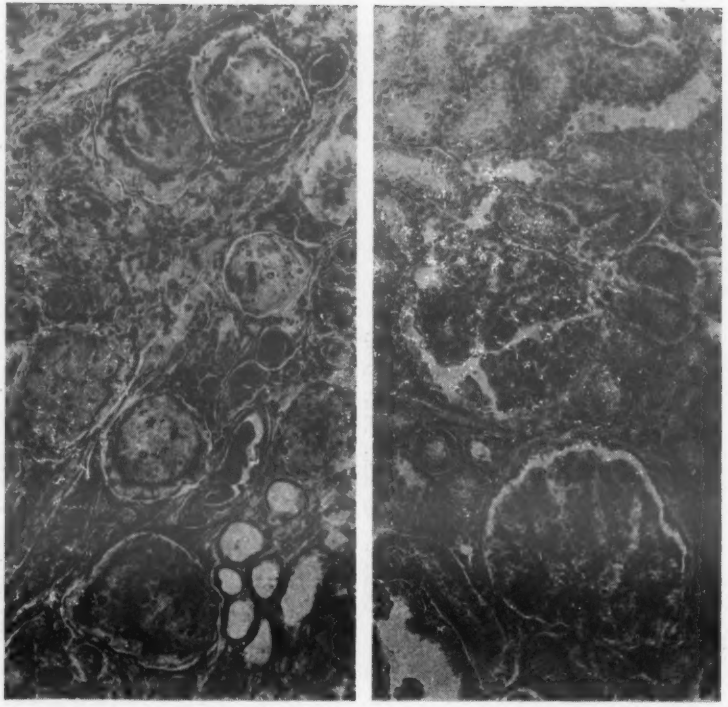
We have utilized dogs to which, prior ethereal anaesthesia, a dose of 80 mg of alloxan per kg of body weight was injected rapidly in the vessels.

Glycemia was immediatly determined after the extraction of blood, and determination was made by the method of Folin-Wu. The samples of blood were obtained after a period of 10 hours of fast.

We have divided in three groups the dogs, which were treated as follows:

1º — In order to exclude eventual actions due to anaesthesia, 5 dogs were anaesthetized with ether and intravenously injected with a solution of alloxan of the same concentration that in the remaining experiments.

2º — In 6 dogs, previous laparotomy and liberation of renal pedicles in both sides, a continuous pressure clamp for vessels was placed in the hilum of the right kidney. On the opposite side —left pedicle— after dissection of the branches in which the renal artery is divided before penetrating in the kidney, an injection of alloxan was performed in one of these arterial branches. Fifteen seconds later, the totality of the left renal pedicle was clamped and the circulatory exclusion maintained during 10 minutes.



A B

FIG. 2. — *Microphotographs of kidney sections of a dog of group II. A) Kidney in the vessels of which alloxan was injected. It presents an intense sclerosis. B) Opposite kidney, excluded by clamping of its vascular pedicle. Normal.*

A B

FIG. 3. — *Fragments of pancreas of dog obtained after the intra-aortic injection with a 50 % solution of Indian ink. A) Control dog. B) Dog with both renal pedicles clamped.*

3º — In 6 dogs the hepatic hilum was clamped during 10 minutes, and meanwhile the alloxan was injected in the saphena vein.

All the surviving animals were killed and the autopsy performed with extraction of fragments of liver, kidney and pancreas for their histological study.

RESULTS

All the control dogs had intense diabetes. Except number 1, all the others died within a lapse of 10 days (table I). In all of them the histological study demonstrated the existence in the liver, pancreas and kidney of the usual lesions of alloxan intoxication.

The animals of the second group did not become diabetic within the 18 days following the injection of a certainly diabetogenic dose of alloxan. The results of this experiment are shown in table II.

TABLE IV

Weight of the kidneys in the dogs of experiment II.

Dog	Kidney Weight		Percentage of weight loss
	Right	Left	
	g	g	
6	51	22	31
7	56	20	60
8	80	24	57
9	46	21	76
11	32	42	8

The clamping of the vascular pedicle of the hepatic hilum had the same protecting effect, as none of the animals thus treated showed any signs of diabetes within the 17 days following the operation. The results are summarized in table III.

The surviving animals were killed and autopsied one month and a half and two months after having been treated with alloxan.

Of the 6 dogs belonging to the second group only 5 could be autopsied. In 4 of them an atrophy of the left kidney was found in the vessels of which alloxan had been injected. The atrophy was of variable intensity, but always very evident. Figure 1 and table IV determine with precision its magnitude.

The histological study demonstrated the existence of a sclerosis in the injected kidney that contrasts with the normal state of the one clamped previously to the injection of alloxan (figure 2).

The histological examination of the pancreas of the dogs belonging to the second and third groups demonstrated the existence of isolated and scarce lesions in some islets.

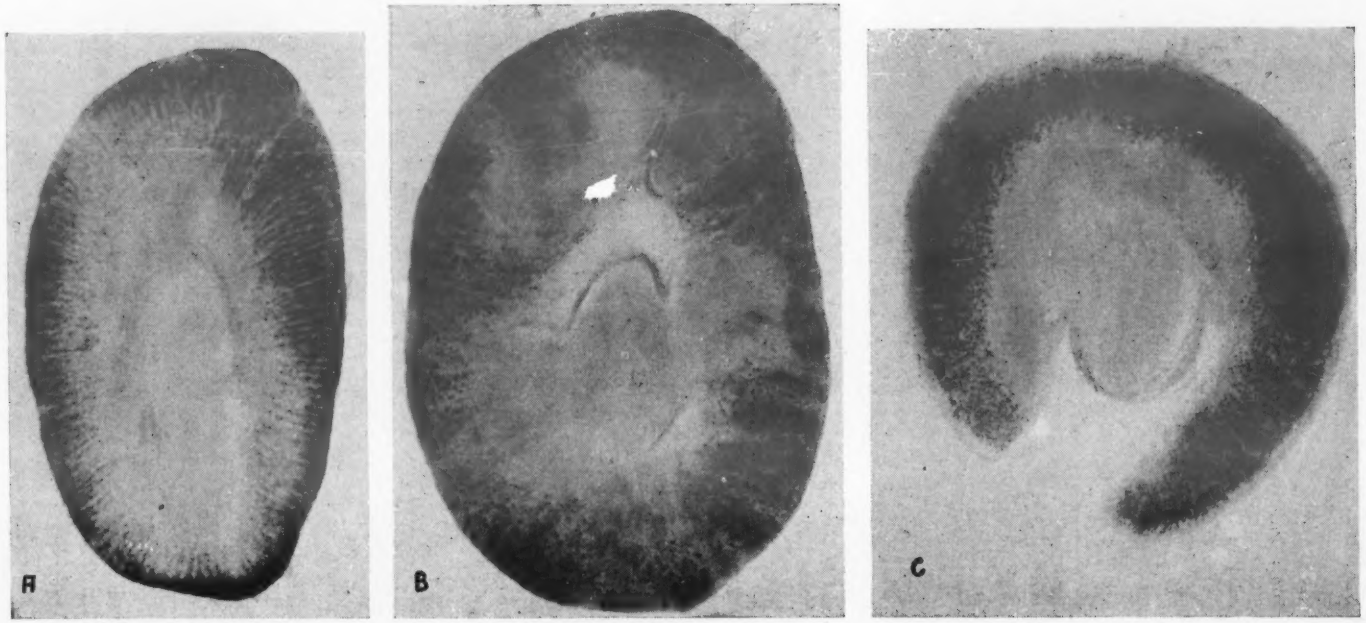


FIG. 4. — *Fragments of kidney of dogs intra-aortically injected with a 4 % solution of soluble Berlin blue. A) With one renal pedicle clamped and the other one free. Cutting thickness 100 micras. B) With the hepatic vessels closed by clamping. Cutting thickness 180 micras. C) With the splenic pedicle closed. Cutting thickness 500 micras. The cuttings, without dyeing, prior dehydration, have been made transparent with benzol and mounted on balsam of Canada.*

In order to prove eventual circulatory changes in pancreas and kidney, 12 dogs, after anaesthesia and artificial respiration, were injected with a 4 % water solution of soluble Berlin blue or a 50 % water solution of Indian ink. The dye was injected in the aorta by means of an ureteral catheter number 12 introduced by the carotid or femoral arteries.

Four control dogs were injected without clamping their renal vessels. Four dogs received the injection with both pedicles clamped and in other four dogs the injection was performed after clamping the vessels of the liver and spleen.

To investigate the eventual existence of a Trueta phenomenon in the animals of the second group, other four dogs were injected with soluble Berlin blue after clamping the vascular pedicle of one kidney, leaving the other free. The injection was performed as in the former series.

In the control dogs injected with Indian ink, the pancreas showed macroscopically a more intense and uniform colouring than that observed in those animals in which the vascular pedicles of both kidneys were clamped (fig. 3).

When either the renal, liver or spleen pedicles were clamped no complete or intense cortical renal ischaemia was found as described by Trueta. Even when the ischaemia was observed in a few glomerules most of them contained the dye (fig. 4).

Discussion

The existence of a renal injury provoked by alloxan does not seem necessary for the development of its diabetogenic action. In the dogs of our second group the renal injury was produced in the injected kidney and notwithstanding they did not become diabetic.

The histological study of the kidneys showed that the renal injury was not due to vascular lesions caused by the handling necessary for injection and liberation of the pedicle. The lesions had a sclerotical charater: they were diffuse, and sclerosed nephrons alternated with normal ones. Segmentary necrosis with the typical characters of infarct was not found.

On the other hand, the clamping of one renal pedicle did not hinder the contact of alloxan with the other kidney. The injections of dyes that we have performed show that the injected substance enters into the cortical glomeruli. We have not been able to demonstrate the Trueta phenomenon in our dogs.

In our experiments even though alloxan circulates through the kidney and causes a renal injury, the dogs did not become diabetic when the renal pedicle was clamped. The protection, in this case, may be produced by a reflex originated at the level of the nerves of the renal pedicle, and would act by a mechanism not yet precised, producing circulatory changes in the pancreas.

The results of the injection in the aorta of Indian ink performed in control animals, and in others with their renal vascular pedicles clamped, afford at the same time a confirmation of the findings of Houssay and Martínez (4) and an argument in favour of their hypothesis.

The same protective effect is obtained by clamping the vessels of the hepatic hilum. The animals thus treated did not become diabetic. The injection of soluble Berlin blue in the aorta of animals with the splenic or hepatic hilum clamped, demostrated the integrity of the cortical circulation of kidney, and therefore the argument of the lack of contact between alloxan and kidney or its smaller intensity cannot be maintained as an explanation of its protective effect.

The fact that the protective action of renal clamping is not specific and can be duplicated by clamping the hepatic or splenic pedicle is in favour of its reflex origin.

SUMMARY AND CONCLUSIONS

The injection of a dose of 80 mg of alloxan per kilogram of weight in the renal artery of dogs with prior clamping of the vascular pedicle of the opposite kidney, produced a severe renal injury (in 4 dogs out of 5 which were autopsied) ; however the animals did not become diabetic.

The clamping of the hepatic pedicle protected also the dogs against the diabetogenic action of a high dose of alloxan.

The clamping of one renal pedicle, or of liver or spleen pedicles did not produce changes in the intrarenal circulation, like those described by Trueta.

The clamping of the renal pedicles caused circulatory changes in the pancreas. It has been proved, through injection of Indian ink in a 50 % water solution that the dyeing in the pancreas of the animals with clamped kidneys was less intense and more irregular than in control animals.

The existence of a renal injury provoked by alloxan is not necessary for the development of alloxanic diabetes.

The protection resulting from the temporary circulatory exclusion of the kidneys is not due to lack of renal injury, but is a consequence of a reflex action on the pancreatic circulation.

REFERENCES

(1) DE OYA, J. C., JIMÉNEZ DÍAZ, C., GRANDE COVIÁN, F.: *Rev. Clín. Española*, 1950, *39*, 318.
(2) DUFF, G. L., WILSON, D. C., MAC MILLAN, G. C.: *Nature*, 1947, *159*, 575.
(3) GOLD, A.: *Nature*, 1947, *159*, 574.
(4) HOUSSAY, A. B., MARTÍNEZ, C. A.: *Rev. Soc. Argent. Biol.*, 1948, *24*, 55.
(5) JIMÉNEZ DÍAZ, C., DE OYA, J. C., GRANDE COVIÁN, F.: *Rev. Clín. Española*, 1946, *21*, 328.
(6) JIMÉNEZ DÍAZ, C., GRANDE COVIÁN, F., DE OYA, J. C.: *Rev. Clín. Española*, 1949, *32*, 173.
(7) JIMÉNEZ DÍAZ, C., GRANDE COVIÁN, F., DE OYA, J. C.: *Bull. Inst. Med. Res. Madrid*, 1948, *1*, 35.
(8) MARTÍNEZ, C., GITTER, S., COVIÁN, M. R.: *Rev. Soc. Argent. Biol.*, 1947, *23*, 81.